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ABSTRACT:

This invention provides methods to produce target cell populations which comprise primitive stem cells, progenitor cells, precursor cells, cells at intermediate stages of differentiation and terminally differentiated cells from almost any living cell within a patient such as a human. These target populations can be used in cell transplantation therapy such as bone marrow transplants, peripheral blood transplants, as well as in the recreation or supplementation of any organ or organ system such as the hematopoietic system. In addition, target cell populations are also useful in gene therapy for the introduction of genes and their expressed products into a patient either systemically or in a targeted fashion.

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(54) Title: SELECTIVE CELL PROLIFERATION

(57) Abstract

This invention provides methods to produce target cell populations which comprise primitive stem cells, progenitor cells, precursor cells, cells at intermediate stages of differentiation and terminally differentiated cells from almost any living cell within a patient such as a human. These target populations can be used in cell transplantation therapy such as bone marrow transplants, peripheral blood transplants, as well as in the recreation or supplementation of any organ or organ system such as the hematopoietic system. In addition, target cell populations are also useful in gene therapy for the introduction of genes and their expressed products into a patient either systemically or in a targeted fashion.

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SELECTIVE CELL PROLIFERATION

Cross-Reference to Related Application

This is a continuation-in-part application of U.S. Serial No. 08/009,593, filed January 27, 1993.

Background of the Invention

1. Field of the Invention

This invention relates to methods and compositions for the production and use of a desired or target cell population. Target cell populations include populations of stem cells and their differentiated progeny from systems such as the hematopoietic system which gives rise to lymphoid and myeloid cells, as well as the various organ systems, tissues, and supporting cellular matrices. Target cell populations of the present invention are useful for cell transplantation, as a therapy or prophylaxis against disease or infection, for reconstitution of a deficient or missing cell population, as a means for introducing genetic information into the patient, and as a cure for congenital or acquired genetic defects and other abnormalities.

2. Description of the Related Art

The hematopoietic system is responsible for supplying all of the morphologically and functionally recognizable cells of the human blood system. The fully mature cells of the blood circulatory system include the erythrocytes, platelets, neutrophilic granulocytes, monocytes, eosinophilic granulocytes, basophilic granulocytes, B-lymphocytes and T-lymphocytes. A striking feature of blood cells is their relatively brief life span. This requires the constant regeneration of the total blood pool throughout life.

The current accepted model of the physiologic system used by the body to constantly produce the many distinct blood cell types is the stem cell model of hematopoiesis. In this model, fully mature cells are replaced, on demand, by morphologically recognizable dividing precursor cells. The precursor cells are of distinct lineages for respective mature cells such as erythroblasts for the erythrocyte lineage,

myeloblasts for the promyelocyte lineage, and megakaryocytes for the platelets. The precursor cells derive from yet more primitive cells that have been classified as progenitor and stem cells. This definition is derived from the functional properties of the cells that are responsible for the production of the more differentiated cell lineages. Some of these cells are the precursor cells of one distinct lineage, such as the erythrocyte progenitor cell called the burst-forming unit - erythrocyte (BFU-E) and the-burst forming unit - megakaryocyte (BFU-MK). Others are multi-potential cells such as the colony-forming unit-granulocyte/macrophage (CFU-GM), responsible for the granulocyte and macrophage lineage cells, and the colony-forming unit - lymphocyte (CFU-L) responsible for both the T- and B-lymphocyte lineage cells. A lineage map of the hematopoietic system is depicted in Figure 1.

As currently viewed, hematopoiesis ultimately derives from a pool of undifferentiated cells called stem cells. The most primitive stem cells are defined by two functional criteria: the potential to self-renew and thus maintain the stem cell pool and the capacity to give rise to progeny that are the committed precursors for all single and multiple hematopoietic lineages. The most primitive stem cells are believed to be extremely rare, being no more than 1 in 10^4 in bone marrow cells and most likely rarer than 1 in 10^6. Therefore the entire pool of the most primitive pluripotent stem cells in the body is probably no greater than 1 to 2 x 10^6 cells. This pool of undifferentiated stem cells gives rise to more differentiated bone marrow stem cells by division and differentiation as well as the progenitor and precursor cells. A progenitor cell, which is morphologically indistinguishable from a stem cell, is committed to one or more specific cell lineages and a precursor cell is committed to a specific cell lineage. Ultimately, through a catenated sequence of proliferation and differentiation, these rare stem cells regenerate the entire hematopoietic system. This process goes on continually throughout life, producing more than 10^{11} cells per day in the human adult.

Stem and progenitor cells together make up a very small percentage of the total nucleated cells in the bone marrow, spleen, and blood. These cells are about ten times less frequent in the spleen than in the bone marrow and even less frequent in the blood. Most of the mononucleated cells (MNC) in the blood, the so-called white blood cells,

are neutrophils (the majority) and lymphocytes comprising greater than about 92% of the MNC. A liter of blood contains about 7.5 x 10^9 white blood cells and about 240 x 10^9 platelets.

The membranes of cells of the hematopoietic system contain distinct receptor proteins that have been used to classify and identify different cell types in terms of their morphological and functional phenotypes. A large number of these membrane receptors have been identified and used as antigens against specific monoclonal antibodies. Many of these antigens are found in only one specific lineage of cells. In particular, specific combinations of antigens have been found to be useful to identify specific cells and lineage of cells in correlation with cell morphology and/or function. One antigen, CD34, has been found on the most primitive progenitor and stem cells found in both the bone marrow and the blood (U.S. Patent No. 4,714,680). This antigen is developmental-stage specific and not lineage-dependent. It appears on normal human bone marrow and blood cells, on colony-forming cells for granulocytes-macrophages (CFU-GM), on colony-forming cells for erythrocytes (BFU-E), on colony-forming cells for eosinophils (CFU-Eo), on multi-potent colony-forming cells for granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM) and on immature lymphoid precursor cells. In human bone marrow, this antigen appears on about 1.8% of normal marrow cells and on about 0.2% of normal peripheral blood cells. CD34 does not appear on normal, mature human lymphoid or myeloid cells. Therefore, the CD34 antigen is useful for the identification of early progenitor and stem cells of the human hematopoietic system. Some of the antigens used for the diagnosis and classification of the hematopoietic system are shown below:

Antigen	Distribution	Antigen	Distribution
CD1	Thymocytes	CD19	Early B cells
CD2	T cells	CD20	Pan B cells
CD3	Pan T cells	CD21	Mature B cells
CD4	Helper T cells, monocytes	CD22	Pan B cells
		CD23	Activated B cells
CD5	Pan T cells	CD33	Early myeloid cells
CD7	Pan T cells	CD34	Stem cells
CD8	Cytotoxic/	CD38	Activated T cells,

	suppressor T cells		plasma cells
CD9	Early hematopoietic cells	CD41	Platelets
CD11c	Monocytes	CD45	Leukocytes

Because hematopoietic progenitor and stem cell populations express the CD34 antigen, anti-CD34 antigen antibody has been used to select subpopulations of hematopoietic cells for research and therapeutic purposes. It was found that transplants containing bone marrow cells that lack the CD34 antigen (34-) fail to engraft, whereas transplants of a small number of CD34+ cells produce engraftment. Other antigens expressed on cells committed to myeloid and lymphoid lineages, together with antigen specific antibodies have been used to select for a population of cells that is not only 34+, but lineage negative (lin-). The 34+/lin- cell population in bone marrow comprises less than 0.5% of the total mononuclear cell population and is even a smaller percentage in peripheral blood. Further, selection of yet a smaller subset of 34+ cells, by isolating cells failing to express HLA-DR (DR-), CD38 (38-) and CD45 (45-) as well as failure to stain with the dye Rhodamine 123 (Rho dull), produces the most primitive stem cells. These cells represent about 0.01% to 0.1% of the bone marrow mononuclear cells, presumably at a lower percentage in the peripheral blood, and morphologically appear as small hypogranular lymphocytes. Cells with these attributes are generally found in the Go phase of the cell cycle and display the capacity to differentiate into cells of recognizable myeloid and lymphoid lineages in in vitro culture, in the severe combined immunodeficient (SCID) mouse model and in other animal systems.

Several techniques are used in clinical and research laboratories to identify classes of cells and to select subsets of cells of the hematopoietic system. One technique is fluorescent activated cell sorting (FACS). In this technique, a multicolor flow cytometer is used to detect and separate cells bound with fluorescent conjugated antibodies to the specific antigens that identify the development stage or lineage stage of the cells of the hematopoietic system (see Fig. 1). Briefly, detectable fluorescent signals are generated by hitting the cells with a laser beam as they pass through a flow sheath. A nonfluorescent forward scatter signal is used to represent volume and a side

scatter signal detects cellular texture and granularity. The color signals of the fluorochromes used to conjugate with the antibodies detect the cell specific antigens. FACS analysis is generally able to analyze two or three colors simultaneously, and from this data, computer programs generate contour plots, dot plots, histograms, perform statistical analyses and the like. Some flow cytometers have sorting capability to isolate and gate sub-populations of cells physically from the sample being analyzed with greater than 95% purity. Most flow cytometers are able to analyze particles as small as 0.5 μm and to detect cell membrane receptors with a density of about 2000 molecules per cell. By such means cell populations that are 34+ and subpopulations such as 34+/DR-/33-/19-/3-/38- can be detected and analyzed, thereby enabling the identification of stem/progenitor cell transplants that are rich in the cells most useful for ensuring both long-term engraftment and short-term hematopoietic recovery.

Other techniques are also used to isolate 34+ and 34+ subpopulations of cells using the physical separation technique of immunoseparation. Antibodies against specific receptor molecules are used in conjunction with immunoaffinity columns or incubation containers to bind cells having the specific target receptor while, theoretically, cells which do not have the target receptor remain unbound. The cells are physically removed from the antibody complex by employing physical fluid shear forces. With an affinity column, cells are flushed from the column into a separate container while in the case of an incubation device, the non-bound cells are first flushed from the device and the bound cells are physically removed by fluid shear into a separate device. By such means, it is possible to select and enrich in concentration specific cell populations such as 34+/lin- which are particularly desirable for stem cell transplants. However, non-specific binding of cells to the affinity antibodies can cause contamination by cell populations of undesired cell types and can reduce the efficiency of cell selection.

Stem cell transplants are used to treat a number of diseases and disorders comprising the groups: hematopoietic malignancies, malignant solid tumors of non-hematopoietic origin, immune disorders, and diseases resulting from failure/dysfunction of normal blood cell production/maturation. Among these are congenital disorders, including severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome,

Fanconi's anemia, congenital red cell aplasia, lysosomal storage disease, cartilage-hair aplasia, thalassemia major, aplastic anemia, leukemias, acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and other hematologic melanomas, lymphoma, multiple myeloma, hairy cell leukemia, malignant histiocytosis, myelodysplastic syndromes, solid tumors such as breast carcinomas, and neuroblastomas.

Three sources of hematopoietic cells are used for therapeutic stem cell transplants, bone marrow, peripheral blood, and cord blood. The most commonly used transplants are bone marrow transplants for the restoration of hematopoiesis in cancer patients who receive high-dose chemotherapy and/or ablative radiation therapy or in patients who have defective hematopoiesis. Peripheral blood transplants are now being advanced as an alternative, less costly, less invasive technique to bone marrow transplants.

Cord blood has also recently been used successfully for the hematopoietic reconstitution of children with lethal disorders of hematopoiesis, as an alternative to marrow-derived cells. Cord blood is obtained from the umbilical cord and placental blood of newborns which is otherwise discarded. Cord blood contains about the same number of progenitor stem cells as does adult bone marrow per unit number of mononucleated cells (U.S. Patent No. 5,004,681).

Three types of stem/progenitor cell transplants are used, syngeneic (identical twin) transplants, allogenic transplants, and autologous transplants using the recipient's own cells. Syngeneic transplants are not rejected and do not cause graft-versus-host-disease (GVHD). Allogeneic grafts, taken usually from a human leukocyte antigen (HLA)-identical donor, are the most common form of transplant used by about 30-40% of patients having a suitable donor. Patients that receive transplants from a phenotypically-matched identical parent or related donor but mismatched for only an HLA-A, -B, or -D locus have transplant results comparable to those receiving transplants from an HLA-identical sibling. Transplants are less successful for recipients mismatched for two or more HLA loci. A smaller number of patients receive transplants from unrelated, HLA-identical donors. In North America, less than 30% of

patients are able to find an HLA-matched sibling and only 3-5% have a one-locus mismatched relative. Therefore, most patients using an allogeneic transplant must find an HLA-unrelated volunteer. Recent studies on unrelated donor transplants have shown that even a single base-pair mismatch in the DNA coding for the HLA loci can lead to clinical complications. Polymerase chain reaction (PCR) assay methods are now being investigated as a possible means to determine true, proper HLA-matchings. Autologous bone marrow transplants are sometimes used to treat patients who do not have an HLA-identical sibling or cannot find a suitable HLA-matched donor.

The traditional bone marrow transplant (BMT) was developed initially as a treatment for disorders of the bone marrow, including leukemia and aplastic anemia. At one time, bone marrow was thought to be the only tissue where the pluripotent stem cells reside and hence bone marrow transplant was necessary to replace these cells when the marrow was lethally ablated by radiation therapy and/or high-dose chemotherapy. It is now known that these stem cells are also present in the peripheral blood.

Bone marrow is collected by an invasive surgical procedure requiring general anesthesia. About one to two liters of bone marrow is extracted with a large-bore needle inserted into a large bone, almost always the pelvis in an area just distal to the iliac crest. One hundred or more separate aspirations are usually required. The marrow is then purified to remove most of the extraneous cells and blood. After purification the marrow is frozen and stored for future BMT reinfusion.

In the case of bone marrow transplants for the treatment of malignancies, after the extraction procedure the patient is subjected to ablative chemotherapy and/or ablative radiation therapy in an attempt to rid the patient's body of all tumor cells. The cells are often treated with cytotoxic chemicals, selected to kill preferentially any tumor cells. In this procedure, the patient's bone marrow cells are essentially killed leaving the patient hematopoietically deficient. The patient's own marrow is then thawed and reinfused. In the case of an allogeneic transplant, the donor's own marrow is used and infused. The marrow cells then engraft, usually within 3 to 4 weeks, and establish hematopoiesis. During this time period the patient is required to undergo intensive and expensive hospital care requiring repetitive platelet transfusions to prevent episodes of

bleeding caused by cytothrombopenia. In addition, during this period, the patient is susceptible to serious and sometimes fatal infections because of neutropenia.

Serious complications often arise from transplantation therapy, in addition to side effects, that lead to patient morbidity and mortality. In the case of bone marrow transplant, about 200 ml of whole marrow are infused into the patient's circulatory system. This large volume causes side effects in patients including hemoglobinuria, red urine, nausea, elevated blood pressure, fever, chills, vomiting and tachycardia. Early complications of bone marrow transplants include chemotherapy and radiation toxicity, graft rejection, GVHD, immune deficiency, infections, and interstitial pneumonitis. Interstitial pneumonitis is also caused by drug and radiation toxicity, as well as by opportunistic infections caused by post-transplantation immunodeficiency. Opportunistic infections from bacterial and fungal organisms are also a complication. The major concern in autologous transplants is the possible reintroduction of malignant cells with the cryopreserved marrow. Different methods are used to attempt to purge tumor cells from the marrow prior to infusion, including density centrifugation, monoclonal antibodies, and pharmacologic techniques.

Morbidity and mortality from GVHD are major problems. GVHD occurs when, in an allogeneic transplant, the donor's T-cells recognize the recipient's cells as foreign and attack them. Post-transplant immunosuppressive treatment with drugs (methotrexate, cyclosporine A) is administered to prevent or minimize GVHD. T-cell depletion ex vivo before transplantation is sometimes used to prevent GVHD. While this approach decreases the risk of GVHD, it is associated with increased graft failure and recurrent leukemia.

Instead of infusing whole marrow, therapeutic approaches have recently been developed to separate stem/progenitor cells from extracted bone marrow. In this case, only the much smaller population of stem/progenitor cells is infused in a volume of about 10 ml. Using this technique the amount of reinfused cells can be reduced by as much as 100-fold, greatly reducing the morbidity associated with whole-marrow transplantation.

In peripheral blood transplantation, stem cell isolation is also used to enrich the transplant with stem/progenitor cells. Peripheral blood stem cell transplantation is less invasive to the patient than the bone marrow aspiration. Cells are collected from a patient by leukaphoresis during which the patient is connected to a centrifugation device used to separate the white blood cells and return the red blood cells and platelets continuously to the patient. Peripheral blood is sometimes also enriched in stem/progenitor cells by mobilizing progenitor cells in the patient's blood using cytokines such as G-CSF and GM-CSF, prior to extraction of the blood. By these means, it is possible to provide progenitor stem cell levels in the peripheral blood about equal to levels found in bone marrow. Benefits of primed peripheral blood stem cell transplants with progenitor stem cell separation/enrichment include the ability to screen out suppressor T-cells in allogeneic transplants to reduce GVHD. In addition, since it is believed that all or most tumor cells are not part of the stem/progenitor cell population, by using positive selection of progenitor stem cells there is also the benefit of improved purging of tumor cells.

In the clinical environment, the level of progenitor stem cells is usually assayed using colony-forming assays for lineage progenitor cells, the most common assays being colony-forming units-granulocyte macrophage (CFU-GM) and burst-forming units-erythrocytes (BFU-E). CFU-GM and BFU-E assays are used to correlate the number of progenitor stem cells required to obtain successful engraftment of transplants. The review of the literature shows that successful engraftment correlates against CFU-GM (U.S. Patent No. 5,004,681). The range of CFU-GM per transplant is 2-425 x 10^4 per kg body weight. An acceptable minimum level is greater than 10 x 10^4 CFU-GM per kg body weight per transplant.

Extensive studies have been conducted to expand hematopoietic stem and progenitor cells, the CD34+ cells, with various degrees of success. The basic approach in most of these studies involves (a) enrichment of CD34+ cell by positive and/or negative absorption to a purity of greater than 80%, (b) culture of cells in basal medium, for example, RPMI-1640 or Iscove's modified DMEM containing fetal calf serum and on a stromal layer as feeder cells, and (c) supplement of a cocktail of

cytokines which include IL-1, IL-3, IL-6, colony stimulating factors (CSFs), erythropoietin (EPO), and stem cell factor (SCF). More recently, some studies have been carried out in serum-free medium without stromal cells which achieved comparable results in cell expansion.

Based on these studies, several observations have been made. First, there is expansion of CD34+ and progenitor cells. Up to a 100-fold cell expansion has been reported for 12 to 14 days. It is likely that the variation in cell expansion is donor-dependent. Second, during culture, cell differentiation towards the myeloid lineage is indicated, leading to a mainly monocyte and macrophage population in long-term culture. Finally, the percentage of CD34+ cells in the total cell population drops precipitously to less than one percent in about 14 to 21 days of culture. These results indicate that there is presently a limited capacity, using current culture conditions, to expand CD34+ cells, which differentiate toward myeloid lineage.

Summary of the Invention

The present invention overcomes the problems and disadvantages associated with current strategies and designs and provides a new method for rapidly producing a desired or target cell population.

Using the method of the present invention, from a very small sample of cells or tissue, large populations of target cells can be easily, inexpensively, and rapidly produced. Target cells can be maintained in culture, expanded, stored for later use, or stimulated to further differentiate as needed. The procedures described herein are short, straightforward, and applicable to many different cell types. Target cell populations are useful in transplantation therapy and provide a number of advantages over current procedures. Multiple pools of donor cells are not necessary, nor are the extensive separation procedures presently required to enrich an inoculum with stem/progenitor cell populations. Moreover, large numbers of stem cells, progenitor cells, and precursor cells for nearly any particular tissue can be produced, as it is these cells which are most useful in transplantation therapy.

As broadly described herein, the present invention is directed to a method for producing an expanded cell culture containing an enriched fraction of a desired target

population of cells and further, to the expanded target cell population produced thereby. The cell culture is enriched in the target cell fraction by culturing the cells in the presence of a balanced selective factor mixture (BSFM) which provides a balance of stimulatory and inhibitory effects that favors the proliferation of the target cell population. The target population comprises a population of primitive stem cells, progenitor cells, precursor cells, cells in intermediate stages of differentiation, terminally differentiated cells or mixtures thereof. These target cells are subsequently useful in transplantation therapy.

The present invention also is directed to the BSFM composition comprising a mixture of cell factors having a balance of stimulatory and inhibitory effects which favors the proliferation of a desired cell population. The composition is produced by treating a cell population with an inducing agent which preferably comprises a mitogen. Useful mitogens include plant lectins such as phytohemagglutinin (PHA) or concanavalin A (ConA), T-cell mitogens such as TPA or mezerein, or a T-cell monoclonal antibody such as OKT3. The BSFM can be selectively modified by removing or adding specific factors to favor the proliferation of a different target cell population. Alternatively, a BSFM can be prepared from a variety of different starting cell populations, thereby creating a BSFM-1, a BSFM-2, and so on, each specific for a different target cell(s) population.

In another embodiment, the present invention is directed to a method of transplantation therapy wherein primary mammalian cells (e.g. from blood, other bodily fluids, or tissues) are cultured in vitro with a BSFM, according to the above-described process. The resulting expanded target cell populations are maintained or cryopreserved for later use or can be immediately introduced into a patient for transplantation therapy or for other therapeutic or prophylactic uses. Target cells may be used to repair or create new organs or organ systems to be transplanted, and can be utilized to supplement existing diseased or damaged organs, tissues, and cell systems. Alternatively, target cells can be employed to produce useful cell products such as hemoglobin from erythrocytes.

In a further embodiment, the present invention provides a method of gene therapy. Target cells are made by the method of the invention and directly transfected

with a genetic sequence or infected with recombinant viral vectors containing a genetic sequence. Cells which have integrated and properly expressed the sequence of interest are selected, isolated, and cultured in vitro. These recombinant cells are then reintroduced into the patient. Useful genes for gene therapy include genes whose expression products are absent or defective in the patient, and genes and other genetic sequences whose expression provide a beneficial effect to the patient.

One further embodiment of the present invention provides a method of transplantation therapy or prophylaxis wherein a patient is treated with BSFM in vivo to selectively proliferate a particular target population of the body. In vivo BSFM therapy can be employed to create or repair organs or cell systems which have become or are expected to become diseased or injured. This form of therapy is relatively non-invasive and currently unavailable for such a wide variety of cells.

Brief Description of the Drawings

Figure 1 The hematopoietic chain and its correlation with developmental-specific antigens.

Figure 2 Diagrammatic representation of the methods which may be used to produce various populations of target cells with a BSFM.

Figure 3 A representative micrograph showing the colony-forming units of a BSFM treated PBMNC culture.

Figure 4 A representative micrograph showing the burst-forming units of a BSFM treated PBMNC culture.

Figure 5 A representation micrograph of unfractionated cells one day after BSFM treatment (10x).

Figure 6 A representative micrograph of unfractionated cells three days after BSFM treatment (10x).

Figure 7 A representative micrograph of fractionated cells one day after BSFM treatment (10x).

Figure 8 A representative micrograph of fractionated cells three days after BSFM treatment (10x).

Figure 9 A representative FACS analysis of CD34+ CD38 DR cells from a unfractionated PBMNC culture after six days of incubation.

Figure 10 A representative FACS analysis of CD3+CD33CD34 cells from an unfractionated PBMNC culture after five days of incubation.

Description of the Invention

Current techniques of stem cell transplants require invasive and costly methods to provide sufficient quantities of stem/progenitor cells to reconstitute fully the patient's depleted or missing cell system. Using the method of the present invention, in a very short time and at low cost, large numbers of cells can be produced allowing for not only the complete reconstitution of the depleted or missing cell system, but also the flexibility of having sufficient cells to permit multiple or cyclic treatments if more than one is deemed necessary. Also, current techniques typically require the purification of cells from very large pools of biological fluid. Using the method of the present invention, large numbers of cells can be produced from very small samples of biological fluid. This saves the expense and time of using inefficient separation techniques to enrich a target cell population, overcomes the risk that a contaminant exists in one of the many samples used to create the pool from which the relatively few stem cells are collected, and even more importantly, provides a consistent source which one of ordinary skill in the art can modify to provide the highest level of a desired product.

1. Target Cell Selection

The method of the present invention is broadly applicable to the selective expansion of an extremely diverse population of cell types. Accordingly, the first step in this method comprises the selection of a desired target cell or mixture of target cells. In the practice of the method described herein, one or more cell types present in an original cell population can be preferentially expanded to enrich the fraction of the target cell(s) in the expanded cell population. Thus, a cell type present at very low levels in normal cell samples can be selectively proliferated to increase its fraction in the expanded population. During this selective expansion and enrichment, the non-target cells in the population can be allowed to die off, to remain unexpanded or to fall in number and/or proportion in the expanded culture. One of the important clinical

advantages of the method of this invention is that cell populations containing a high fraction of the selected target cell(s) can be produced simply and in many cases without need for separation or purification steps or the addition of separate and expensive cytokines. In some instances, the desired expanded cell population will include a number of cell types whose fraction, relative to physiological cell samples, is increased or the ratios of these targets cells changed to facilitate a particular utility. Where, for example, hematopoietic reconstitution is the objective, a cell population substantially enriched in stem cells and progenitor cells to provide long-term engraftment may also include specifically tailored fractions of precursor cells or terminally differentiated cells to provide for short-term positive effects.

It is also possible, using the method of the present invention, to produce a cell population that includes a substantial fraction of cell types not present in the starting cell population. This can occur by providing conditions that favor the differentiation or dedifferentiation of cells along their known differentiation pathways.

The target cell can be selected from any mammalian cell and preferably a primary mammalian cell and more preferably a primary human cell. A target cell population or the mammalian cell population from which it is selected may comprise an entire cell lineage, organ, or organ system including the stem, progenitor, and precursor cells of that system. Examples of tissues and organs from which such cell populations can be selected include hematopoietic cells, liver cells, pancreas cells, kidney cells, brain cells, nerve cells, heart cells, lymph node cells, thymus cells, spleen cells, bone marrow cells, bone cells, cartilage cells, muscle cells, endothelial cells, epithelial cells and the like. The preferred target cells are hematopoietic cells. Where the ultimate use for expanded cell populations involves tissue repair or regeneration, it will be appreciated that mixed populations of cell types normally found in such tissue can be the desired target cell mixture or can be prepared separately and combined in vivo or in vitro to facilitate such an objective. In a preferred approach, the target cell population includes stem cells and/or progenitor cells which can, either during the preparation of the expanded cell population or during its use, provide differentiated cells in the pathway of the stem/progenitor cell lineage.

In the hematopoietic system, the desired target cells can include pluripotent stem cells of the most primitive type as well as progenitor cells, precursor cells, cells at intermediate stages of differentiation and terminally differentiated cells or mixtures thereof. These cells include myeloid progenitors such as CFU-G (granulocyte), CFU-GM (granulocyte/macrophage), CFU-GEMM (granulocyte/erythrocyte/macrophage/monocyte), CFU-E (erythrocyte), CFU-MK (monokaryocyte), CFU-M (macrophage), CFU-Eo (eosinophil), CFU-Ba (basophil), BFU-E (erythrocyte), and BFU-MK (megakaryocyte), and lymphoid progenitors such as CFU-L (lymphocyte), CFU-B (B cell) and CFU-T (T cell). Also useful are target cell populations comprising T lymphocytes, B lymphocytes and their committed precursors. Myeloid lineage cells such as megakaryocytes (platelets), erythrocytes, granulocytes, macrophages, monocytes, basophils, eosinophils and their committed precursors are also desired targets alone or as mixtures with other target cells.

Of particular interest from the standpoint of use in hematopoietic reconstitution are cells expressing the CD34 antigen. CD34+ cells can include primitive CD34+lin- cells as well as those more primitive cells characterized as CD34+CD38-CD45-DR-RHO123(Dull). Also of interest in treating immune disorders and diseases are cells expressing T cell antigens such as CD3, CD4 (helper T cells) and CD8 (suppressor T cells). Erythrocytes are also a desirable target cell both for use of the cell population in transplant therapy and for use in the production of hemoglobin.

One skilled in the art will appreciate which target cells and target cell mixtures are useful for the applications described herein and other applications employing specifically tailored cell populations.

Cells and cell populations made by the method of the invention are analyzed morphologically, antigenically, and functionally. First, preferentially expanded cell populations are examined microscopically for size, characteristics such as the presence or absence of granules, and overall appearance. Stem cells and other relatively undifferentiated cells are small with well defined membranes, whereas, more differentiated cells are generally larger. Second, stem cells express characteristic antigens which are detectable in assays such as an ELISA or a RIA using antigen-

specific monoclonal antibodies and are sortable by FACS techniques. Results from these antigen-binding assays provide a developmental map of cell lineages. Lastly, cells are also analyzed functionally by their ability to form colonies and/or to perform other biological functions in vitro.

2. Selection of a Starting Cell Population

In the preferred embodiment of the present invention, a first cell population is selected and induced to produce a BSFM which comprises a mixture of cell factors obtained from the first cell population and having a predetermined balance of stimulatory and inhibitory effects which preferentially favor the expansion and enrichment of the desired target cell(s). These first cell populations comprise primary cells of the blood, bone marrow, body tissues of humans or nonhumans, preferably mammalian tissues, or established cell lines. This first cell population can be of the same cell type as the desired target cell, contain cells of the target cell type, contain cells that differentiate to cells of the desired target cell type or cells that are of a completely different type from the desired target cell type. In one embodiment of this invention, this first cell population can be the cell population that is ultimately expanded as described below.

It is generally preferred, however, to employ a separate cell population of the same type to be ultimately expanded. Thus, for the hematopoietic cell expansion, a blood-based cell sample can be conveniently employed. Often it is desirable for the first cell population to comprise a portion or subpopulation of the cell population that will be expanded as described below. It is contemplated, however, that tissue cells of non-hematopoietic origin may be expanded by inducing a first population of hematopoietic cells to produce a BSFM that will selectively expand the target cells in the tissue.

With reference to the hematopoietic system, the first cell population useful to produce a particular target-specific BSFM preferably is selected from peripheral blood cells, cord blood cells and bone marrow cells. Due to numerous clinical advantages, the selection of peripheral blood cell populations is preferred. First, peripheral blood is easy to obtain requiring a relatively noninvasive procedure (needle stick). No hospital stay is required, the risk of infection is very small, and the procedure can be performed

by most health care workers. Second, peripheral blood samples can be taken with little regard to the health of the individual because blood loss to the patient has little to no systemic impact. Third, peripheral blood is already used as a source for a number of medical assays. Standard procedures and supplies are presently available in every medical office.

The peripheral blood cell populations useful as the starting first cell population can include whole peripheral blood (e.g., an as-removed blood sample) and peripheral blood mononucleated cells (PBMNC). The source of PBMNC can include the product of known collection and separation techniques. For example, the buffy coat fraction from a coarse centrifugal separation can be employed as well as finer separation fractions from specific gravity-based systems such as FICOLL. It is also possible to employ as the first cell population highly purified PBMNCs or a specific subpopulation thereof having a particular cell type (e.g., T-lymphocytes) by employing affinity-based separation techniques. As described below, it is often useful to employ PBMNCs that have undergone minimal separation procedures as they may contain useful accessory cells and be less damaged than highly processed cells. The first population of cells can be used shortly after harvest and separation or can be kept refrigerated or frozen for future use.

3. Inducing the First Cell Population

An important aspect of the invention is the ability selectively to expand and enrich target cells in a cell population in the presence of a BSFM. Production of this BSFM is preferably accomplished by inducing the first cell population described above to produce this target-specific mixture of factors. Thus, the inducing step should be controlled to produce the desired BSFM. The inducing step can be performed on the first cell population in a separate step or as a part of the target cell expansion step when the second cell population described below is the same as the first cell population. In a preferred embodiment the first cell population is induced by an added inducing agent and also may be further induced during culture by factors produced by the cells, e.g., in a cascade fashion.

In the preferred embodiment, the target cell(s)-specific endpoint directing nature of the BSFM is influenced by a combination of physical, chemical and biological parameters. One such parameter of particular significance is the nature of the added inducing agent, as described below. Another parameter that can be varied to change the target cell(s) is the nature of the first cell population, as described above. Applicants have found for example, that, in the context of hematopoietic cells, relatively unprocessed, coarsely separated PBMNCs such as those obtained from a leukaphoresis treatment produce BSFMs that are targeted to stem/progenitor cells such as CD34+ cells. Other factors that can influence the BSFM inducing process include culture conditions, e.g., medium, temperature, time, pH and the like.

In one preferred embodiment the inducing step is carried out by culturing a selected first cell population in a medium which supports leukocyte growth, to which has been added an inducing agent. The medium preferably can be serum-free and does not require stromal cell involvement. Suitable medium types include ISCOVES modification of DMEM, RPMI 1640, and CCM-2 produced by Verax Corporation of Lebanon, New Hampshire.

The added inducing agents that are useful according to the preferred embodiment in general comprise materials that have a mitogenic effect on the cell types of the first cell population. Mitogens are known for various cell types and the effects of such mitogens in inducing various factors have been observed for hematopoietic cells. T-cell mitogens are often used for this process.

Among the classes of mitogens that are useful to induce or facilitate the induction of BSFMs from hematopoietic cells are plant lectins, T-cell mitogens, and monoclonal antibodies. Particular plant lectins that have the desired mitogenic activity include those derived from the following:

Lectin Source
Phaseolus vulgaris (PHA, phytohemagglutinin)
Dolichos biflorus
Solanum tuberosum
Sophora japonica
Maclura pomifera
Pisum sativum
Ulex europeus (UEA-I, U. europeus agglutinin I)
Ulex europeus (UEA-II, U. europeus agglutinin II)
Arachis hypogaea
Glycine max
Canavalia ensiformis (Con A, concanavalin A)
Triticum vulgaris (WGA, wheat germ agglutinin)
Ricinus communis (RCA-I, R. communis agglutinin I)
Lycopersicon esculentum
Phytolacca americana (PWM, pokeweed mitogen)
Listeria monocytogenes (LPS, lipopolysaccharide)

A particularly useful group of plant-derived mitogens includes PHA, ConA, mezerein (MZN) and TPA (and related diterpene esters). TPA and some of its related compounds are set forth below:

Phorbol 12-myristate-13-acetate (TPA)
Phorbol (4-0-methyl) 12-myristate-13-acetate
Phorbol (20-oxo-20-deoxy) 12-myristate-13-acetate
Phorbol 12-monomyristate
Phorbol 12, 13-didecanoate
Phorbol 12,13-dibutyrate
Phorbol 12,13-dibenzoate
Phorbol 12,13-diacetate

Mitogens of non-plant origin can also be useful such as Staphylococcal enterotoxin A (SEA), Streptococcal protein A, galactase oxidase and T-cell antibodies such as OKT3. Interferon-alpha (IFNα) and IFNβ can also be used as inducing agents in some circumstances as well as stem cell proliferation factor (SCPF), stem cell factor (SCF), any of the growth factors and the bone morphogenic proteins (BMPs).

In the preferred induction process, the first cell population selected as described above is cultured in the presence of an enhancing (i.e. potentiating) agent prior to the actual inducing step. Agents useful to enhance the induction include TPA (or related

phorbol esters) MZN, IFNα and IFNβ. This step can then be followed by addition of an above-described inducer to the culture. Most preferred combinations involve the use of IFNα and IFNβ, and MZN as an enhancer, and PHA, ConA or OKT3 as an inducer.

Selection of an inducer and/or enhancer from the above-listed agents or their known equivalents can be made without concern for their cytotoxic or tumorigenic properties since the BSFM is preferably separated, as a supernatant, from the treated cells. This step can be performed in a way to ensure that the BSFM is not contaminated with any harmful residual mitogens or by-products thereof. In the case where inducing or enhancing agents such as INFs and T-cell antibodies are employed, the separation of BSFM from cells or cell products/debris may not be necessary.

The amount of inducing agent used and the length of treatment can be empirically determined for each specific cell culture treated. The preferred amount of inducing agent used is from about 5 ug/ml of medium to about 100 ug/ml of medium. More preferably, for the described hematopoietic cell induction process, the inducing agent can be added in an amount of from about 5 ug/ml to 50 ug/ml, most preferably about 20 ug/ml to 50 ug/ml when treating first cell populations of whole or fractionated blood. In those cases where a potentiator/enhancer is employed, it can be added to the medium at a level of from about 5 ng/ml to 500 ng/ml, preferably 5 ng/ml to 50 ng/ml and most preferably 10 ng/ml to 20 ng/ml. Treatment times can be from hours to days, preferably from about 1 day to 10 days and most preferably 2 days to 5 days. Alternatively, treatment may involve multiple treatments over a longer period of time, or more intense treatments over a shorter period of time.

One skilled in the art can select the desired induction conditions, agents and starting cell populations based on the teachings contained herein and simple experiments to correlate changes of initial conditions with target cell effects. The present invention is based at least in part on the discovery that employing an induction factor mixture (BSFM) having a balance of both positive and negative effects permits the controlled selective expansion of any number of desired target cell populations.

The induction step has been described in the preferred <u>in vitro</u> mode, but it is possible to perform this step <u>in vivo</u> in certain circumstances. Thus, in the case where

a cell population (either circulating or static) can be treated with an inducing agent that is not otherwise harmful to the patient, the BSFM can be generated in vivo and either collected from the patient for further use ex vivo or left in the patient to mediate the selective target cell expansion in vivo, e.g., at the site of tissue repair or regeneration. An inducing agent utilized in vivo may be injected or infused in the patient, taken orally, or directly placed on the cells to be treated by, for example, transdermal patch, and may involve a single treatment or multiple treatments.

4. The Balanced Selective Factor Mixture

The present invention provides a family of BSFMs, each designed to act on a particular second cell population in a specific way to cause expansion and enrichment of the desired target cell(s). All of these BSFMs share the characteristics of providing a mixture of cell factors that have a balance of stimulatory and inhibitory effects which preferentially favor the proliferation of the selected target cell(s). Prior approaches have generally focused on the use of defined proliferative (i.e. stimulating) factors in connection with narrowly selected cell types to determine the positive effect of these factors or combinations of factors. The methodology of the present invention is based on the use of a balance of complex positive and negative effects and feedback loops similar to those employed in nature. As a result this method is at the same time both powerful and simple.

By way of non-limiting exemplification, the BSFM which has been shown to preferentially expand CD34+ cells in a PBMNC population can, according to a preferred embodiment, be prepared by inducing a leukaphoresis fraction of peripheral blood with MZN and ConA. This BSFM has been partially characterized and found to contain the following known cell factors:

Cytokine	Cell Source
IL-1	Macrophages
IL-2	T-lymphocytes
IL-3	T-lymphocytes
IL-4	T-lymphocytes
IL-5	T-lymphocytes

IL-6	T-lymphocyte, macrophages, lymphoid cells, monocytes
IL-8	Monocytes
CSFs (GM-CSF, G-CSF)	T-lymphocytes, B-lymphocytes, macrophages, monocytes
INF-γ	T-lymphocytes
TNFs (TNF-α, TNF-β)	T-lymphocytes, macrophages, neutrophils

The concentrations of some of these cytokines have been determined. For example, the concentration of IFN-γ and IL-2 are about 10,000-20,000 IU/ml and about 2000-5000 IU/ml, respectively. The concentration of the other listed cytokines can be readily determined, for example, by ELISA assays, using commercially available assay kits and/or methodologies. Examples of additional cytokines include EPO, SCPF, SCF, any of the growth factors and the BMPs.

Given the teaching of this invention it is possible for one of ordinary skill in the art to characterize fully the factors contained in this BSFM and determine the optimum nature and amount of known active factors (both stimulatory and inhibitory) which are required for expanding the desired CD34+ cells. Accordingly, the synthesis of such optimized, defined BSFM's is within the scope of this invention. In addition, this invention provides a means to identify and isolate new cytokine(s) which are useful or important for expansion of a target cell and/or maintenance of the target cell type. For example, after removing all the known cytokines from the preparation using immunoaffinity techniques, the remaining components can then be tested biologically for their ability to support the expansion and/or differentiation of target cells. In addition, further tests can be conducted by reconstituting the known components to prepare a BSFM of known composition and testing the ability of this BSFM to produce the result of the induced BSFM. If it does not, those skilled in the art could determine the missing factors.

The isolated "unknown" component(s) can also be analyzed and purified by conventional techniques. Thus, the amino acid sequence of proteinaceous component(s) can be determined and the genes for these component(s) can be isolated and sequenced

by conventional recombinant techniques. Consequently, these proteins can be produced recombinantly.

Another embodiment of this invention involves the modification of one BSFM composition by adding or subtracting positive or negative factors to produce a second BSFM which favors preferential expansion of a different target cell(s). Several approaches can be used to obtain BSFM preparations which favor the expansion and enrichment of different or additional target cells. First, one or more of the factors in the BSFM preparation described above can be removed, preferably by affinity chromatography. Affinity matrices with antibodies against specific cytokines are commercially available. For example, if BSFM is allowed to pass through an affinity column to remove IL-2, the resultant BSFM will no longer favor the expansion and enrichment of lymphoid lineage cell types. Also, macrophages can be removed by adherence to plastic surfaces, thereby resulting in a cell population which produces little or no IL-1 or CSFs in the BSFM. Further, known factors within BSFM can be selectively inactivated without isolation using factor-specific monoclonal or polyclonal antibodies. By using a positive- and/or negative-affinity approach, modified forms of BSFM can be obtained to favor expansion of a desired target cell (see Fig. 2). Alternatively, based on the kinetics of cytokine induction, BSFM preparation with various compositions can be obtained by harvesting at different times after induction. For example, IL-2 is induced ahead of IFN-γ. IFN-γ concentration is higher in BSFM preparation harvested at 96 hours than that in BSFM harvested at 48 hours, while IL-2 concentration will be the same in both BSFM preparations.

5. Expansion of Target Cells

The next step in the process of the present invention involves the selective expansion of target cells of a second cell population by culturing this cell population in the presence of the BSFM that selects for the desired target cell. As indicated above, the second cell population can be of a different cell type or the same cell type as the first population. In many cases it will be desirable to employ an original cell population that is divided into first and second identical subpopulations for the generation and use of the BSFM. Under certain circumstances the first and second cell populations may

be the same population, i.e., the same cells are first induced and then expanded, with or without separation steps.

Selection of the desired second cell population depends on the desired target cell. The target cell should be among the cell types in this population or derivable from it, e.g., by differentiation or dedifferentiation. In general, the same kinds of cell types described above for the first cell population can be used for the second population.

The selective target cell(s) expansion can be controlled by varying the nature and amount of the BSFM, and the culture conditions. Selection criteria for BSFMs are discussed above. The culture step is preferably carried out in an appropriate basal medium, which can be supplemented with defined cytokine(s). Culture conditions for individual cell types may vary, but standard tissue culture conditions form the basis of culture treatment. Typically, cells are incubated in 5% CO_2 incubators at 37°C in media. Specific chemical agents, proteins, media components such as insulin or plasma, and certain growth or colony stimulating factors (CSFs) may be required for the maintenance of certain cell types. These requirements are either known in the subject field or can be determined by one of ordinary skill in the art. By way of a non-limiting example, the BSFM that favors CD34+ cell expansion can be cultured in serum-free media such as CCM-2.

The BSFM can be added to the medium in an amount sufficient to obtain the desired expansion/enrichment of the target cell(s). Additive amounts will vary depending on the nature of the BSFM, the make-up of the second cell population and the culture conditions. In practice, this addition can be from about 1% to 10% and preferably is about 2% to 5%.

The length of the culture steps can be varied to assist further in the selective proliferation of the target cell. When the cell population involves cells on or induced to enter a differentiation pathway, the final target cell enrichment may depend on when the culture is terminated. Typically, for example, a population enriched in CD34+ cells can optimally be cultured for about 5 to 20 days and preferably for about 5 to 10 days, depending on the extent of enrichment desired.

Once the desired expanded target cell population is achieved it can be used at that time, or refrigerated or frozen for future use. In an alternative embodiment, the target cell population can be modified to change the population to one enriched in a second target cell(s). For example, a CD34+ rich/lymphoid preferring mixture can be converted into a population enriched in T lymphocytes by further culturing the population in the presence of known factors that promote the differentiation of T-cell progenitors down the lymphoid line. Also, the target cells can be incubated for longer periods of time, or subjected to varied culture media, such as media supplemented with factors to drive the target cells to the desired population and/or the desired stage of differentiation. Examples of factors include IL-2, granulocyte colony-stimulating factor G-CSF, macrophage colony-stimulating factor M-CSF, erythropoietin (EPO), interferon (IFN), retinoic acid, SCPF, SCF, BMP and any of the growth factors such as nerve growth factor (NGF), epidermal growth factor (EGF), fibroblast-derived growth factor (FGF), and platelet-derived growth factor (PDGF). During this step cell cultures may or may not be maintained in BSFM-containing media as conditions warrant.

Apart from obtaining target cells for various clinical applications, one added advantages of this invention is the inherent purging of tumor cells during cell expansion. It has been reported that the number of some chronic myeloid leukemic cells rapidly declines in long-term culture. The purging of tumor cells during expansion according to this invention can be facilitated by the presence of cytokines and cells, for example, IFN-γ, TNFs, and activated macrophages which have antiproliferative and/or cytotoxic effects on tumor cells.

As in the case of the inducement step, the expansion step can be carried out in vivo or in vitro. In vivo applications involve introducing the BSFM into the bloodstream for circulating cell populations or into specific tissue cells. Alternatively, cells can be combined with or treated by the BSFM and then reintroduced into the body to effect repair, reconstitution or regeneration of systems, tissue or organs.

According to one embodiment, this invention is directed to the establishment of a hemostasis for a given cell culture by mimicking the "natural" control of in vivo biological events. The definition of hemostasis is limited to the dynamic equilibrium

established as a result of cell/cell and cell/factor interactions under specific culture conditions. Hemostasis can be attained by providing a balanced mixture of factors and an array of various cell types in the culture. The balanced mixture of factors is added exogenously and additional factors can be induced de novo during culture. For example, addition of IFN-γ and/or TNF-α to the culture can induce the production of CSFs from monocytes and macrophages. As a result of the specific cell/cell and cell/signal interactions, a balanced stimulatory and inhibitory environment is created to favor the population's expansion of and/or differentiation towards a target cell type. This dynamic equilibrium may change as the cell population changes as a result of expansion and enrichment of a target cell type. Accordingly, the initial balanced stimulatory and inhibitory environment can be modified in order to maintain a continued expansion of the target cell.

6. The Expanded Cell Population

The expanded cell population of the present invention can have both a greater number of target cells (expansion) and a higher percentage of target cells (enrichment) in the final population, as compared to the original population. For example, cells present in very low numbers and fractions, such as stem cells, can be expanded and enriched to give populations having greater than about 5%, preferably greater than about 20% and most preferably greater than about 50% of said target cells. For cells originally present in larger numbers or for applications requiring high fraction populations (e.g., erythrocytes for hemoglobin production), expanded populations containing at least about 50% target cells and preferably at least 80% target cells can be produced according to the present invention. In these new populations, the target cell can be expanded to at least about 500-fold, preferably at least about 1,000-fold, and most preferably at least about 10,000-fold.

As an example, one enriched cell population according to the present invention is the hematopoietic cell population comprising about 30% CD34+ cells. This population also has about 7.5% of CD34+CD38-CD45-DR-Rho123 (Dull) cells. These cell populations are enriched for CD34+ cells by at least about 1,500 fold over normal peripheral blood samples.

7. Uses of the Expanded Cell Population

Expanded target cell populations made by the method of the present invention are useful for cell transplantation, tissue regeneration including the regeneration of organs or cell systems such as bone marrow replacement, supplementation therapy, such as specific cell infusion, and as a means for treating or preventing an infection or a disease.

Preferably, target cells of the present invention are stem cells, progenitor cells, or precursor cells. Stem cells, progenitor cells, and precursor cells are more easily utilized to create more differentiated cells, whereas, the reverse is often not true. In one embodiment, target cells are used for the regeneration or replacement of an entire cell system, such as the hematopoietic system or of cells of only the lymphoid or myeloid lineages. Erythroid target cell populations can be stockpiled or stored and used for blood transfusions. Alternatively, erythroid cells can be used for the generation of large amounts of red blood cell expression products such as hemoglobin. Lymphoid cells can be used in the treatment of diseases such as Bruton's agammaglobulinemia (B cell deficiency), DiGeorge's syndrome (thymic hypoplasia), SCID (adenosine deaminase deficiency), thrombocytopenia such as Wiscott-Aldrich syndrome, and other immunological deficiency diseases.

In general, peripheral blood stem cell transplantation therapy, according to this invention, can be substituted for currently used transplantation procedures such as bone marrow transplants and peripheral blood transplants. With either of these procedures, large amounts of bone marrow or peripheral blood are required, the collection processes are very invasive to the patient, and the stem cells are of poor quality because of the extensive manipulations required for purification.

In contrast, peripheral blood transplantations by the current invention have no such disadvantages. First, only a small sample of peripheral blood is required which is obtained by a very low-invasive process. Second, no purification steps are necessary. A concentrated population of autologous stem cells is produced in a single culture. Moreover, the cells produced can be specifically tailored as required or the entire

hematopoietic system can be reconstituted. This combination of advantages is not possible using currently available technology.

In accordance with this aspect of the invention, target cell populations of the hematopoietic system can be used as a therapy and often a cure for certain diseases. Briefly, a sample of the desired cells may be obtained from the affected patient. Optionally, the patient may be primed before the sample is taken by administering a priming agent such as G-CSF or a chemotherapeutic agent. These cells are treated, if necessary, to remove any diseased cells and cultured according to the method of the present invention. The patient is then treated to destroy all affected cells of the body with, for example, high-dose chemotherapy or radiation treatments, after which cultured cells are reintroduced. Provided the patient can be cleared of all affected tissue, even for a short period of time, complete destruction or removal of the affected cell system or organ becomes a viable option.

Infections and diseases which are localized to a particular cell type or cell population can also be treated with the methods of the present invention. This includes the anemias such as red cell membrane disorders, red cell enzyme deficiencies, disorders of hemoglobin synthesis, isohemagglutinins, erythroblastosis fetalis, malaria, mechanical or chemical trauma to red blood cells, hypersplenism, sideroblastic anemia, anemia related to chronic infections, and myelophthisic anemias due to marrow infiltrations. This also includes diseases of the immune system such as the non-Hodgkin's lymphomas including the follicular lymphomas, Burkitt's lymphoma, adult T-cell leukemias and lymphomas, and acute leukemia. Additional diseases also treatable by the methods of this invention are neoplasias such as breast cell carcinoma, testicular cell carcinoma, the solid tumors, neuroblastomas, neurofibromas, melanomas, ovarian cancer, pancreatic cancer, liver cancer, stomach cancer, colon cancer, bone cancer, squamous cell carcinomas, adenocarcinoma, prostate cancer, and retinoblastoma, and nutritional diseases such as malabsorption syndromes, vitamin deficiencies and obesity.

As indicated above, the present invention is equally applicable to non-hematopoietic cell systems. Target cells produced by the method of the invention can be used for tissue regeneration, organ replacement or supplementation, cell

transplantation, or as a prophylactic or therapeutic against disease and infection. For example, in tissue regeneration, target cell populations of undifferentiated cells of a particular tissue can be created. Liver, kidney, nerve, pancreas, skin, and bone cells may be readily and rapidly produced in large quantities. First, BSFM is cultured with a population of cells to create primitive stem cell populations. These primitive cells are then treated with specific factors such as, for example, nerve growth factor, fibroblast-derived growth factor, GM-CSF, or erythropoietin, and other agents to produce the desired tissue.

In one embodiment, according to the methods of the present invention, skin can be effectively and safely produced and transplanted to a patient. First, a small sample of healthy epithelial cells is surgically removed from a patient and placed in tissue culture. Next, the BSFM is prepared from this same sample of cells, from another epithelial sample or other tissue sample taken from the same patient earlier, or from a cell culture obtained from another source. The patient's epithelial sample is then cultured in the presence of an effective quantity of BSFM. From the resulting enriched and expanded culture, large numbers of epithelial precursor cells can be made to proliferate and produce natural skin which is grafted onto the patient. When using the patient's own cells as a sample, the risk of rejection is very low or absent, and new epithelial cells are very rapidly produced. For patients in need of such therapy, for example burn patients, speed is of the essence as the risk of life-threatening secondary infections is quite severe.

In another embodiment, it is also possible to create entire organ systems from progenitor cells. For example, a small sample of pancreatic cells is obtained from a patient or a healthy, immunologically matched donor. A BSFM composition is prepared using this same sample of pancreatic cells which may be Islets of Langerhans cells, epithelial pancreatic cells, hematopoietic cells or cells from another source. The pancreatic cells are then treated with an effective amount of BSFM which expands and enriches the culture in stem, progenitor, precursor or differentiated pancreatic cells. From these cells, a pancreas, or at least a portion of a pancreas, (e.g. Langerhans cells including the A, B, D, and PP cells) can be recreated and surgically reintroduced to the

patient. These cells will not be immunologically rejected and will function in the same manner as natural cells and produce insulin. Consequently, using the method of the present invention, in certain instances, type I diabetes (insulin dependent diabetes) could be cured, and, at the very least, complications attributed to type II diabetes (insulin resistant diabetes) could be alleviated. This method can also be used to produce islet cell populations for incorporation into implantable devices where they function under regulation to produce insulin.

Cell populations produced by the method of the present invention also can be used to supplement, repopulate, or totally recreate cell systems such as the kidney, liver, gall bladder, colon, lung, selected muscle tissues, nerves, selected veins, arteries and capillaries, tendons and ligaments and the cells of the gastrointestinal system. It is one aspect of this invention that cells and cell populations created can be utilized for whole organ reconstruction, utilizing methods currently available and known to one of ordinary skill in the art such as those disclosed in U.S. Patent No. 5,032,508, which is hereby specifically incorporated by reference.

Cell populations according to the present invention can also be used as a therapeutic agent against disease or infection. Cell populations to be transplanted can be screened and treated for infections such as viruses and bacteria, which can be effectively and completely eliminated using procedures which cannot be utilized in vivo. For example, a small sample of a patient's fluid or tissue containing the infected cells can be purged of infection and the cell numbers amplified by culturing with BSFM. In vitro procedures for the selective destruction of diseased cells in transplantation therapy are known. Briefly, the cell sample is placed in culture and treated with, for example, an antiviral agent such as a reverse transcriptase inhibitor (e.g. 3'-azido-3'-deoxy-thymidine (AZT)). BSFM treatment may be performed before, after or concurrently with antiviral treatment. Alternatively, the cells may be cloned and individually screened to select for uninfected cells and the resulting population expanded. At the same time, the patient is treated with high doses of chemical agents, drugs, or radiation to eliminate all infected cells in the body. Once the disease is eliminated from the

expanded cell sample and the patient, the expanded cell sample is then reinfused into the patient, recreating a total or partial cell system.

Infections caused by viruses such as the human immunodeficiency virus (HIV) which causes the acquired immune deficiency syndrome (AIDS) can be treated in this manner. A small sample of blood is removed and its T-cell populations amplified according to the method of this invention. All traces of virus are then eliminated from the sample using procedures which are well known in the art and the patient is treated with high doses of antiviral agents (e.g. AZT) to destroy all virus particles and, if necessary, other agents to destroy virus-infected cells. These steps are not possible using current transplantation technology because secondary infections would set in and kill the patient before transplanted cells were established and functioning. According to the method of this invention, the patient can be infused with a sufficient amount and variety of cells to reconstitute an active immune system. Alternatively, an AIDS-infected patient can be continuously treated with a fresh supply of uninfected T-cells which had been cleansed of virus and expanded from a sample of the patient's blood according to the method of the present invention. Although this may not eliminate the virus, the patient may never develop AIDS-related complications, remain symptom-free, and be able to lead a relatively normal life. Additionally, having the patient in a stronger physical condition may allow for more aggressive therapeutic measures which may clear the infection.

In another embodiment, cell populations prepared by the method of the invention could be used prophylacticly against disease or infection. For example, a sample of blood could be taken from a patient and treated to expand a population of stem/precursor cells. These cells are then treated in a manor to select for the expansion of pre-B cell populations. These pre-B cells are treated with antigens to a particular disease which could be a viral infection from influenza, herpes simplex virus I or II, cytomegalo virus, the human T-cell leukemia viruses (e.g., HIV), polio virus, rhinovirus, respiratory syncytial virus, hepatitis A, B, and C viruses, measles virus, and JC virus, a bacterial or fungal infection from an organism such as Staphylococcus, Streptococcus, Mycobacterium, Clostridium, Neisseria, Enterobacter, Pseudomonas,

Salmonella, Treponema, Candidae, and Aspergillas or a parasitic infection such as amebiasis, pneumocystis, malaria, trypanosomiasis, the holminthic diseases and sarcoidosis.

The target cells are treated with antigen in a manner to stimulate antigen specific antibody production and the target cells are injected back into the same or a different patient. That patient would have an antibody resistance to the particular disease chosen without ever having been exposed to the infection or a vaccination. In a similar fashion, any cell type of the immune system could be treated to produce a cellular or humoral resistance to infection or disease. Alternatively, using this method it may also be possible to treat these same diseases and infections may be treatable.

Another embodiment of the present invention is directed to a procedure for providing a effective means for genetic therapy. In view of the relative ease by which cell populations can be removed and cultured, one of ordinary skill in the art can also introduce a genetic element into the cells prior to their reintroduction into the patient. This can cure many of the deficiency disorders which can be attributed to a single missing or defective genetic element. In addition, hematopoietic stem cells lend themselves very well as a vehicle for disseminating the missing product to various parts of the body. Hematopoietic stem cells as well as other cells produced by the method of the invention can also be used for targeting a specific recombinant product to a specific area of the patient's body, for example, by targeting an anticancer agent to hepatic cells in patients with primary carcinoma of the liver, by targeting cancer suppressor gene products to specific cells in patients with retinoblastoma, or by targeting anti-plaque forming enzymes to hematopoietic cells in patients with atherosclerosis.

Examples of other candidate diseases for gene therapy according to the method of the present invention include hemophilia A (glucose 6-phosphate hydrogenase deficiency), familial hypercholesterolemia (e.g., cholesterol 7-alpha-hydroxylase deficiency), thalassemia, sickle-cell anemia, cystic fibrous, Tay-Sachs disease (G_{M2}-gangliosidosis), glycogen and lysosomal storage diseases (e.g., sphingolipidoses, mucolipidoses, Wolman's disease, Pompe's disease, Gaucher's disease), and SCID, to name just a few.

Procedures for genetic therapy are known in the art. Briefly, a sample of cells is removed from the patient and placed in culture according to the method of the invention. The sample is maintained in culture treated in a manner in accordance with the present invention to favor the expansion of a fraction of the cell population which includes the stem cells. These cells are then subjected to techniques for the introduction and stable incorporation of genetic elements. Introduction of genetic elements may be accomplished before, during, or after cell expansion according to the method of the present invention. Incorporation of genetic elements during expansion may be preferred in some cases. Examples of these techniques include transfection, such as calcium-mediated or microsome-mediated transfection, cell fusion, electroporation, microinjection, or infection using recombinant vaccinia virus or retrovirus vectors. These vectors would contain functional genetic elements which express products, such as, adenosine deaminase for the treatment of SCID. Cells which acquire the genetic element are selected, further expanded and reintroduced to the patient. These cells circulate throughout the body and supply adenosine deaminase, or some other product, where needed.

Another preferred example for this approach is in the treatment and possible cure of diabetes. A sample of pancreas tissue from an insulin-dependent diabetic patient is biopsied and placed in tissue culture. The sample is cultured with BSFM to selectively proliferate stem cells, progenitor cells, precursor cells or differentiated pancreatic cells. These cells are then transfected with the gene which codes for the expression of insulin. Preferably, the gene is under the control of a promoter which is transcriptionally and translationally regulated in a manner similar or identical to the natural insulin gene, which may be the natural insulin promoter. These recombinant cells are then injected or surgically transplanted back into the pancreas of the diabetic patient. The recombinant cells incorporate into the pancreas and perform the function of pancreatic B cells, which is to supply insulin to the bloodstream as required. This treatment can be repeated as necessary, however, once biopsied, pancreatic cells do not have to be continually taken from the patient, but can be maintained in culture or

stored (e.g. cryopreserved) for later use. These are significant advantages not envisioned by currently available techniques.

Another embodiment of the present invention is directed toward a genetic therapy for treating an infection with cellular transplantation. For example, target cells, preferably hematopoietic cells, could be prepared according to the method of the present invention. These cells are transfected or infected with a recombinant DNA sequence which expresses a therapeutic product, and inoculated back into the host to effect the therapy. Therapeutic products which are useful include antibiotics, anticancer agents including chemotherapeutic drugs, peptides and cytotoxic compounds, and expression products such as antisense RNA and ribozymes. A particularly useful product is the expression product of the multidrug resistance (MDR) gene which, when incorporated into a patient's hematopoietic cells, allows for the use of higher, and consequently more effective, doses of chemotherapeutic agents in the treatment of certain cancers. In view of the fact that the methods of the present invention can selectively expand a desired population of cells, drugs and other agents can be preferentially targeted to certain tissues and organs for a maximum therapeutic effect with a minimum of side effects. In addition, these same procedures could be utilized to introduce genetic elements into a cell to provide resistance to disease or infection as a prophylactic or precautionary measure. These forms of treatment are not possible with current technologies.

Alternatively, target cells could be integrated with a genetic element which expresses an antigenic or immunogenic product. Once the cells are placed back into the patient, an immune response would occur creating circulating antigen specific antibodies and cells to any substance which expresses the antigen such as an infecting organism. In effect, such recombinant cells would constitute a vaccine against the infecting organism.

In another embodiment, a bank of cell cultures is provided comprising multiple populations of stem cells, progenitor cells, or precursor cells produced according to the method of this invention which are able to differentiate into a variety of specific cell lineages. This cell bank can be created for a single individual and placed in long-term

storage by, for example, cryopreservation. Briefly, a sample of cells from various organs and cell systems of an individual are obtained, HLA typed, and cultured according to the method of the present invention with a selected BSFM. Target cell populations which develop are expanded, pelleted by centrifugation, suspended in a cryopreservation medium such as dimethylsulfoxide (DMSO), divided into samples, and deep-frozen. Samples can be thawed and expanded in culture when needed as, for example, when the patient is in need of cell transplantation therapy. Alternatively, the samples could be frozen to create a cell bank, and later thawed and expanded according to the method of the invention only when needed. These approaches are not possible using current technology because large numbers of undifferentiated cells cannot be maintained or expanded in culture.

In a similar fashion, target cell populations can be stockpiled from different individuals creating banks of stem, progenitor, and precursor cells representative of a complete or partial spectrum of, for example, the human leukocyte antigens (HLA) for use in autotologous or allogeneic transplantation therapy. A bank of these cells can be maintained or cryopreserved for each organ, tissue, and tissue system. Candidate patients for establishing such cell banks other than humans include economically valuable mammals such as cattle, sheep, pigs, and horses, domesticated animals such as dogs and cats, zoo and wild animals such as various monkeys and other primates. It is also possible to establish HLA-typed blood and/or tissue sample banks in which the typed samples can be frozen until needed and then expanded using the process of this invention.

In another embodiment, fetal genetic testing can be performed according to the method of the invention. First, a sample of peripheral blood is taken from a pregnant woman which contains a small number of fetal cells. Fetal cells are expanded by the method of the present invention. Separation of fetal cells from maternal cells, either before or after expansion, can be employed if desired, by FACS sorting or similar processes. The resulting expanded population of fetal cells can be easily and reliably genetically tested by procedures which are known and currently available such as by amniocentesis.

The following examples are offered to illustrate embodiments of the present invention, but should not be used as limiting the scope of the invention.

EXAMPLES

Example 1

Expansion of CD34+ Cells

A. Preparation of BSFM

Peripheral blood cells obtained by leukaphoresis were diluted 1:10 in culture medium CCM-2 (Verax Corporation) containing 20 units of heparin/ml. An equal volume of the diluted blood sample was mixed with 2% acetic acid and the total number of mononucleated cells estimated using a hemocytometer. In general, the number of mononucleated cells from a leukaphoresis unit was about 30 to 50 x 10^6 cells/ml.

To induce BSFM, the peripheral blood cells were diluted to a final concentration of about 4 x 10^6 mononucleated cells/ml in serum-free CCM-2 medium containing 20 units/ml of heparin. A total volume of 80 ml of cell suspension in a T-150 flask was pre-incubated with 10 ng/ml of mezerein in a humidified 5% CO_2 incubator at 37°C for about 2 hours. Concanavalin-A was added to a final concentration of 20 μg/ml and the cells were incubated at 37°C for about 4 days. The supernatant was harvested and the cell debris was removed by centrifugation. The clarified supernatant was stored at 4°C or frozen at -20°C before use.

B. Optimizing BFSM Addition Level

The optimum potential of BSFM level for expanding a peripheral blood cell(s) population was examined in this example using the BSFM as prepared in example 1A. Unfractionated and ficoll-fractionated peripheral blood cells were used. The ficoll-fractionated blood cells were prepared by diluting 10 ml of peripheral blood from a leukaphoresis unit with 20 ml of CCM-2 medium containing 20 units/ml of heparin and 10% total calf serum. 15 ml of the diluted blood samples were loaded onto 10 ml of Ficoll-Hypaque in a sterile 50 ml culture tube. The cells were centrifuged in a table-top centrifuge at 400 x g for 30 minutes at room temperature (25°C). Cells were harvested from the aqueous ficoll interphase and washed twice in CCM-2 medium

containing 10% fetal calf serum. The cells were then resuspended before use in serum-free CCM-2 medium or CCM-2 medium containing 10% FCS.

Unfractionated cells were diluted to a final cell density of about 2 x 10^5/ml in CCM-2 medium containing 1% to 5% BSFM as indicated and in the presence or absence of fetal calf serum. One and one-half mls of cell suspension per well were suspended in a 24-well plate and incubated at 37°C in a 5% CO_2 incubator. The cell count and viability were determined daily for 5 days. As indicated in Table 1, the highest rate of proliferation was observed in CCM-2 medium containing 5% BSFM. Further studies confirm that the rate of cell proliferation peaks in CCM-2 medium containing 5% BSFM. There was no difference between the rate of cell proliferation in medium containing 5% or 10% BSFM. In addition, the cells have been observed to proliferate equally well in both serum-free and serum-containing medium. The ability of the CCM-2 medium containing 5% BSFM to support the proliferation of ficoll-fractionated PBMNC was comparable to that for unfractionated PBMNC.

TABLE 1

Proliferation of Unfractionated PBMNC

DAY	TOTAL CELL COUNT (10^5/ml)			
	0% BSFM*	1% BSFM	2% BSFM	5% BSFM
0	3.00	3.00	3.00	3.00
1	5.75	5.55	4.75	5.65
2	4.36	4.96	5.96	6.88
3	4.20	5.45	6.00	6.16
4	6.35	5.55	7.40	8.60
5	6.80	7.00	5.40	7.00

* The basal medium is CCM-2 containing 10% FCS.

C. Expansion of Peripheral Blood Mononucleated Cells

The expansion of the PBMNC cells was studied by culturing both unfractionated and fractionated cells in CCM-2 medium containing 5% BSFM, 10 ng/ml of recombinant human stem cell factor (SCF) and 1 unit/ml of recombinant human erythropoietin (EPO) in a 24-well plate. Each well was seeded with 1½ ml of cell suspension with a cell density of 1 to 2 x 10^5 mononucleated cells/ml. The cell count and viability were estimated after 5-6 days of incubation. At the end of the incubation, the cells were subcultured in the same culture condition with a seeding density of 1 to 2 x 10^5 cells/ml for another 5 to 6 days. From the initial cell culture, the cells were subcultured twice. As indicated in Table 2, the fold increase of cell numbers exceeded 600 in both unfractionated and ficoll-fractionated cell cultures for a period of 16 days with two passages.

TABLE 2

TOTAL PBMNC EXPANSION

CULTURE*	TOTAL FOLD OF INCREASE	
	Fractionated	Unfractionated
Initial	7.6	4.8
1st Passage	62.2	67.7
2nd Passage	613.1	602.7

* Total of 16 days.

D. Analysis of the Cell Population During Expansion

The composition of the hematopoietic cells in the total cell population was analyzed by three methods: FACS analysis to determine the expressed surface markers, e.g. CD34; colony-forming assay to estimate the total number of progenitor cells, specifically BFU-E and CFU-GM in the cell population; and morphological analysis by light microscopy.

The list of surface markers used for FACS analysis is summarized in Table 3. In most instances, three color stainings were used to assign and determine the subpopulation of CD34+ cells in the culture (Table 4). For example, three separate antibodies vs. CD34, CD38, HLA-DR respectively were used to determine the cell type which expressed the listed, if any, markers as a means to determine the CD34+ cell subpopulations. Other combinations included CD34, CD33 and CD3 to monitor differentiation into lymphoid and myeloid lineage cells.

TABLE 3

CD MARKERS USEFUL FOR CHARACTERIZING SUBSETS OF CELLS

MARKER	DESCRIPTION
CD3	Pan T-Cell
CD4	Helper T-Cell
CD8	Cytotoxic/Suppressor T-Cell
CD19	Early B-Cell, B-Cell Specific
CD33	Early Myeloid Cells
CD34	Stem Cells, Progenitor Cells
CD38	Activated T-Cells, Early Progenitor Cells
HLA-DR	Activated T-Cell, Monocytes, B-Cell

TABLE 4

CELL MARKER COMBINATIONS

CD34 / CD33 / CD19
CD3 / CD4 / CD8
CD34 / CD3 / CD33
CD34 / CD38 / DR

To determine the total number of progenitor CFU-GM cells obtained in the culture, 1 x 10^5 mononucleated cells were suspended in 1 ml of CCM-2 medium containing 20% FCS, 0.9% methyl cellulose, and 10 ng/ml of recombinant human GM-CSF and plated in a 35 mm tissue culture dish with grid. The colonies formed were scored after 14 days of incubation in a 5% CO_2 incubator at 37°C. To determine the number of erythroid progenitor cells, BFU-E, 1 x 10^5 mononucleated cells were placed in a medium as described in the CFU-GM assay except that recombinant GM-CSF was replaced with 1 unit/ml of recombinant human erythropoietin. Again, the total colony forming units were determined on Day 14 of incubation by light microscopy.

To study the cell morphology, cytospin staining technique was used. Briefly, the cells were concentrated by centrifugation and the cell smear was stained with Wright's staining. Cell morphology was analyzed by light microscopy.

E. Expansion of Myeloid and Erythroid Progenitor Cell

In a 24-well plate culture, ficoll-fractionated PBMNCs were seeded at a cell density of about 2 x 10^5/ml and 1.5 mls/well. The cells were cultured in CCM-2 medium containing 5% BSFM, 10 ng/ml recombinant human SCF and 1 unit/ml of recombinant human erythropoietin for 5 days. The total CFU-GM and BFU-E progenitor cells in the Day 0 culture as well as Day 5 culture were estimated, using colony-forming assay as described previously. Representative CFU-GM and BFU-E colonies are shown in Figures 3 and 4 respectively. As indicated in Table 5, there was a 32.3-fold increase in CFU-GM and a 237.5-fold increase in BFU-E progenitor cells

after 5 days of incubation. For this example, the total cells in the culture increased by about 5-fold after 5 days of incubation, indicating a preferential expansion of CFU-GM and BFU-E cells or CD34+ precursor cells thereof.

TABLE 5

EXPANSION OF PROGENITOR CELLS

DAY	PROGENITOR CELLS / 10^5 CELLS			
	TOTAL CELL*		FOLD OF INCREASE	
	CFU-GM	BFU-E	CFU-FM	BFU-E
0	3	2	–	–
5	97	475	32.3	327.5

* Total CFU-GM and BFU-E were 950 cells/ml and 4,655 cells/ml, respectively.

F. Expansion of CD34+ Cells

The selected BSFM prepared as described in Example 1A was intended for the expansion of CD34+ cells which favor lymphoid lineage differentiation. Accordingly, both unfractionated and ficoll-fractionated PBMNC were cultured in CCM-2 medium containing 5% BSFM, 10 ng/ml recombinant human SCF and 1 unit/ml recombinant human erythropoietin in a 24-well plate as described previously. The cells were subcultured on Day 5 and Day 11 and the surface marker, CD34 was determined by FACS analysis on cells harvested on Day 0, Day 5, Day 11 and Day 16. As indicated in Table 6, CD34+ cells increased from less than 0.5% to greater than 70% of the cell population. Figures 5 and 6 show the 1 and 3 day, respectively, cultures for unfractionated cells. Figures 7 and 8 show the 1 and 3 day, respectively, cultures for fractionated cells.

TABLE 6

CD34+ CELL ANALYSIS

DAY	% of Total Cells	
	Fractionated	Unfractionated
0	<0.5	<0.5
5	55-63	[1]
11	50-52	73-75
16	35-36	74-75

[1] Not Done

G. Analysis of CD34+ and CD3+ Cell Subpopulations.

In a separate test to analyze the CD34+ and CD3+ cells subpopulations, both unfractionated and fractionated cells were cultured as described previously in CCM-2 medium containing 5% BSFM, 10 ng/ml SCF and 1 u/ml erythropoietin in 24-well plate for 5 and 6 days respectively. The rate of PBMNC proliferation is summarized in Table 7.

TABLE 7

PBMNC Proliferation

	UNFRACTIONATED CELL		FRACTIONATED CELL	
DAY	TOTAL COUNT[1]	FOLD OF INCREASE	TOTAL COUNT[1]	FOLD OF INCREASE
0	0.33	---	0.17	---
5	1.47	4.4	---	---
6	---	---	1.50[2]	8.7

[1] $x10^6$/ml

[2] Viability is 87.2%

To determine both the CD34+ and CD3+ cell subpopulations, both the unfractionated and fractionated PBMNC were subjected to FACS analysis based on the following surface markers: CD34, CD38, HLA-DR, CD3, CD33, CD4, CD8. The results of the analysis are summarized in Table 8. The total CD34+ cells in the unfractionated and fractionated cell culture are 28.2% and 36.5% of the total cell population respectively. In addition, based on CD38, DR and CD3 surface markers, an array of CD34+ cell subpopulations, ranging from more primitive to more committed progenitor cells were obtained during culture. Some representative FACS analyses are shown in Figures 9 and 10.

TABLE 8

FACS ANALYSIS[1]

SURFACE MARKER	% OF TOTAL CELL POPULATION			
	UNFRACTIONATED		FRACTIONATED	
	DAY 0	DAY 5	DAY 0	DAY 6
CD34+CD38+DR+	0	1.6	0	0.6
CD34+CD38+DR-	0	3.5	0	0.8
CD34+CD38-DR+	0	7.8	0	13.5
CD34+CD38-DR-	0	15.3	0	21.6

[1] Peripheral blood cells from Subject 6.

Example 2

Preparation of Modified BSFM

A modified BSFM was obtained by the protocol described in Example 1.A. Five mls of modified BSFM were passed through an anti-IL-2 affinity column containing 1 ml of matrix. The flow-through fraction (the modified BSFM) was used for the preparation of culture medium for myeloid lineage cell expansion. The culture medium contains CCM-2 medium, 5% modified BSFM, 10 ng/ml of recombinant human SCF and 1 u/ml of recombinant human erythropoietin.

Two x 10^5 PBMNC/ml were seeded in a 24-well plate with 1.5 mls of cell culture per well. The cells were subcultured every 5 to 6 days for 2 passages. At the end of

each of 5 to 6 days of incubation, total cell count and viability were determined. The composition of the cell population related to the expression of CD34, CD33, CD19, CD3 markers were analyzed by FACS. In addition, colony-forming assays were performed on the cells obtained from cultures. The results indicate expansion of CD34+ cells, the population of which consists mainly of myeloid lineage cells (CD33+) with various degrees of differentiation.

Example 3

Expansion of Lymphoid Lineage Cells

The expansion of lymphoid cells was studied by culturing unfractionated and fractionated PBMNCs in a 24-well plate. Both the culture conditions and medium, are described in Example 1. The cultures were passed twice for a total of 16 days of incubation. On day 0, day 5, 11, and 16, the CD3+, CD4+ and CD8+ cell populations were analyzed by FACS. As indicated in Table 9, the CD3+, CD4+ and CD8+ cell populations were expanded and maintained. After 16 days of incubation, the total cell populations in both unfractionated and fractionated cell cultures expanded in excess of 600-fold.

TABLE 9

Analysis of CD3+ , CD4+ and CD8+ Cells

DAY	% of Total Cells					
	Unfractionated			Fractionated		
	CD3+	CD4+	CD8+	CD3+	CD4+	CD8+
0	75	--	--	75	--	--
5	--	--	--	76	54	17
11	74-80	--	--	70-77	--	--
16	88	65	22	84	37	57

The CD3+ cells in both unfractionated and fractionated cell cultures after 16 days of incubation were further analyzed by FACS to determine the subpopulations of CD3+ cells with respect to CD4 and CD8 markers. The results of the analysis are summarized in Table 10 and indicate that these cell populations were expanded and maintained in culture.

TABLE 10

ANALYSIS OF CD3 CD4 CD8 CELLS

	% of Total Cells	
Cell Markers	Unfractionated[1]	Fractionated[1]
CD3+CD4+CD8+	3.8	2.1
CD3+CD4+CD8-	52.0	28.7
CD3+CD4-CD8+	16.7	45.3
CD3+CD4-CD8-	15.0	7.5

[1] Day 16 culture which has been subcultured twice on Day 5 and Day 11. The total percentages of CD3+ cells in the total cell population from both unfractionated and fractionated cell cultures was 84 and 88 respectively.

Example 4

Expansion of Erythroid Lineage Cells

A cell density of 2×10^5 cells/ml of both unfractionated and fractionated PBMNC is used to seed a 24-well plate with 1.5 ml of cell culture per well. The culture medium consists of CCM-2 medium containing the BSFM described in Example 2, 10 ng/ml recombinant human SCF and 10 u/ml recombinant human erythropoietin and 50 millimolar ferric citrate.

The cell cultures are incubated at 37°C and subcultured every 5 to 6 days. At the end of each of the 5 to 6 days of incubation, the total cell count and viability are determined. The cell population is analyzed by FACS and morphological observation using light microscopy. The results indicate expansion of erythroid lineage cells, leading

to a cell population containing proerythrocytes, erythrocytes, and highly differentiated erythroid cells.

Example 5

Reconstitution of the Hematopoietic System

To test the ability of the CD34+ cells obtained from expansion in culture to reconstitute the hematopoietic system, both unfractionated and fractionated PBMNC are cultured in CCM-2 medium containing 5% BSFM, 10 ng/ml recombinant human SCF and 1 u/ml recombinant human erythropoietin for 5 days as described in Example 1. The cells are harvested and concentrated by centrifugation in a table-top centrifuge at 200 x g for 20 minutes at room temperature. About 5 x 10^6 cells in 0.1 to 0.2 ml of phosphate buffered saline, pH 7.4, from each of the unfractionated and fractionated cell culture are injected intraperitoneally (IP) into a SCID mouse. Ten weeks after injection, cells from under the kidney capsule in the SCID mouse are analyzed by FACS for evidence of specific cell incorporation. The results indicate that the cultured CD34+ cells can engraft an intact functioning human hematopoietic system in SCID mice.

Example 6

Preparation of BSFM

To demonstrate a potentiator and an inducer other than those described in Example 1 to induce a BSFM which favors expansion of CD34+ cells toward lymphoid lineage differentiation, 4 x 10^6 mononucleated cells/ml are pre-treated with 300 IU/ml of IFN-β for two hours. The cells are incubated for another 4 days after the addition of 10 μg/ml of PHA. The ability of the BSFM to expand PBMNC is tested as described in Example 1, using 24-well plate cultures. The results demonstrate that the BSFM obtained by using a potentiator/inducer combination different from the one used in Example 1 supports and expands a comparable CD34+ cell population.

Example 7

Expansion of Islet Cells

Porcine pancreatic islets are isolated and islets with average diameter of about 150μ are obtained by density gradient centrifugation using Ficoll. About 10,000 islets are plated on a 60 mm petri dish in the presence or absence of 1 ml of collagen matrix. The culture medium consists of DMEM, 20% of horse serum, 5% BSFM as described in Example 1, and 1% hypothalamus extract. The cells are incubated at 37°C on a rotating platform. On Day 0, Day 7, Day 14, and Day 21, islet cells cultured in the presence or absence of collagen matrix from duplicate petri dishes are assayed for total cell count and viability. In addition, the concentrations of insulin and glucagon in the culture media are determined by radioimmunoassay as a means to demonstrate the proliferation of A and B cells. The results indicate the proliferation of islet cells and the intact functionality of both the A and B cells as indicated by the production of insulin and glucagon.

Example 8

Cell Expansion Using Cells from Different Donors

PBMNC's were separately collected from 6 human donors and were specially Ficoll-fractionated using the protocols described in Example 1.B A BSFM was obtained by the protocol described in Example 1.A.

Cells from each donor were cultured in 24-well plates using one and one-half mls of cells suspension per well at initial seeding densities of 4 x 10^5 and 1 x 10^6 cells per ml. The cells were incubated at 37°C in a 5% CO_2 incubator for 5 to 7 days.

The cells from each donor were suspended in medium formulations made from CCM-2 basal medium plus the addition of 2% BSFM and/or blood plasma, and/or 10% fetal bovine serum (FBS), and/or selected cytokines: 1 unit/ml of recombinant human erythropoietin (EPO), 10 ng/ml of recombinant human stem cell factor (SCF), 10 ng/ml of recombinant human interleukin-3 (IL-3), 10 ng/ml of recombinant human interleukin-6 (IL-6). The medium formulations used are shown in Table 11.

TABLE 11
Medium Formulations

Medium Formulation	Medium Composition*
#1	2% BSFM, EPO, SCF, Plasma
#2	2% BSFM, Plasma
#3	2% BSFM, EPO, SCF
#4	2% BSFM
#5	IL-3, IL-6, EPO, SCF, Plasma
#6	IL-3, IL-6, EPO, SCF
#7	10% FBS

* The basal medium is CCM-2

For each donor's cells, the cell count and viability were estimated after 5-7 days of incubation. The total numbers of CFU-GM cells and BFU-E plus CFU-E cells at the start and at the end of each culture were determined using the procedures described in Example 1D.

The average total cell expansion (and the standard deviation) for the 6 donors' cells for each of the medium formulations is shown in Table 12.

TABLE 12
Average Total PBMNC Expansion

Medium Formulation (See Table 11)	Total Fold of Increase	
	ISD[1] = 4 x 10^5 cells/ml	ISD[1] = 1 x 10^6 cells/ml
#1	*5.34 (3.18)[2]	2.26 (1.08)
#2	*5.31 (3.63)	2.36 (1.01)
#3	4.62 (0.92)	2.00 (0.67)
#4	4.60 (1.10)	2.10 (0.80)
#5	*1.40 ((1.50)	1.00 (1.20)
#6	0.40 (0.20)	0.50 (.20)
#7	0.70 (0.40)	0.60 (0.30)

*Standard Deviation value is skewed by the value of a single donor.
[1] ISD = Initial Seeding Density of cultures.
[2] Average Value (Standard Deviation) of the values for each of the six donor's cells.

The average expansion of CFU-GM and BFU-E + CFU-E progenitor cells are shown in Tables 13 and 14, respectively.

In Tables 12, 13, and 14, where the value of the Standard Deviation is large compared to the Average Value, this Standard Deviation value is skewed by the data of one donor (which is from two-times to four-times larger than the Average Value of the donors).

TABLE 13

Average Total CFU-GM Expansion

Medium Formulation (See Table 11)	Total fold of Increase	
	ISD = 4 X 10^5 cells/ml	ISD = 1 x 10^6 cells/ml
#1	*11.2 (12.6)	2.3 (1.4)
#2	5.9 (2.2)	2.5 (2.2)
#3	1.6 (1.1)	2.5 (3.7)
#4	*2.9 (4.0)	1.3 (1.4)
#5	5.1 (3.2)	3.5 (1.1)
#6	1.1 (1.8)	0.7 (0.8)
#7	0.4 (0.3)	0.9 (0.5)

* Standard Deviation value is skewed by the value of a single donor.

TABLE 14
Average Total BFU-E/CFU-E Expansion

Medium Formulation (See Table 11)	Total fold of Increase	
	ISD = 4 X 10^5 cells/ml	ISD - 1 x 10^6 cells/ml
#1	3.0 (1.8)	2.4 (2.1)
#2	3.7 (1.3)	2.5 (3.4)
#3	0.6 (0.3)	1.1 (1.5)
#4	0.8 (0.1)	0.9 (1.0)
#5	*10.0 (6.2)	7.9 (5.6)
#6	1.4 (0.3)	0.8 (1.1)
#7	0.5 (0.4)	1.2 (0.8)

* Standard Deviation value is skewed by the value of a single donor.

Example 9

Analysis of Cell Size Distribution

PBMNC's were obtained by leukapheresis and a BSFM prepared as described in Example 1.A. A modified BSFM was prepared by the addition of blood plasma to the BSFM (2% plasma). Ficoll-Hypaque fractionated cells were prepared and cultured as described in Example 1.B. using a CCM-2 basal medium (without FCS) plus 10 ng/ml of recombinant human stem cell factor (SCF) and 1 unit/ml of recombinant human erythropoietin (EPO).

Cell count and viability were measured at the end of 5-6 days of incubation and the cells analyzed by FACS to determine cell size and the subpopulation of $CD34^+$ cells.

Representative FACS analysis forward light scatter (FS) and side light scatter (SS) plots are shown in Figures 11 and 12, respectively, for cells cultured with and without plasma added to the BSFM. Without plasma added to the BSFM the cell size distribution is in two distinct subpopulations as shown in Figure 12. The lower FS subpopulation of cells are smaller size cells and this subpopulation contains the preponderance of cells that are CD34 positive. With plasma added to the BSFM, cells in the smaller size subpopulation are negligible and the number of CD34 positive cells is significantly reduced.

Other embodiments or uses of the invention will be apparent to those skilled in the art from consideration of the specifications and practice of the invention disclosed herein. It is intended that the specifications and examples be consideration exemplary

only, with the true scope and spirit of the invention being indicated by the following claims.

WE CLAIM:

1. A method for producing a cell population with an enriched fraction of at least one hematopoietic target cell which has a desired position in the hematopoietic chain comprising the steps of:

a) treating a first cell population comprising hematopoietic cells to induce said first cell population to produce a balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from said first cell population and having a balance of stimulatory and inhibitory effects which preferentially favors the proliferation of said target cell; and

b) culturing a second population comprising hematopoietic cells for a time sufficient and in the presence of an amount of said BSFM sufficient to expand preferentially said target cells and enrich the fraction of said target cells in said second cell population.

2. The method of claim 1 wherein said target cell comprises pluripotent stem cells.

3. The method of claim 1 wherein said target cell comprises progenitor cells.

4. The method of claim 3 wherein said progenitor cells are myeloid progenitors.

5. The method of claim 4 wherein said myeloid progenitors are selected from the group consisting of CFU-G, CFU-M, CFU-GM, CFU-GEMM, CFU-E, CFU-MK, CFU-Eo, CFU-Ba, BFU-E and BFU-MK.

6. The method of claim 3 wherein said progenitor cells are lymphoid progenitors.

7. The method of claim 6 wherein said lymphoid progenitors are selected from the group consisting of CFU-B, CFU-T and CFU-L.

8. The method of claim 1 wherein said target cells are selected from the group consisting of B lymphocytes and their committed precursors.

9. The method of claim 1 wherein said target cells are selected from the group consisting of T lymphocytes and their committed precursors.

10. The method of claim 1 wherein said target cells are selected from the group consisting of erythrocytes and their committed precursors.

11. The method of claim 1 wherein said target cells are selected from the group consisting of platelets, granulocytes, macrophages, basophils and eosinophils or their committed precursors.

12. The method of claim 1 for producing a cell population with enriched fractions of at least two hematopoietic target cells wherein said target cells comprise pluripotent stem cells and progenitor cells.

13. The method of claim 12 wherein additional target cells comprise myeloid cells and lymphoid cells.

14. The method of claim 1 wherein said target cells are CD34+

15. The method of claim 14 wherein said CD34+ cells are CD34+ lin-.

16. The method of claim 14 wherein said CD34+ cells are CD34+CD38- CD45- DR- RHO 123 (Dull).

17. The method of claim 14 wherein said CD34+ cells are CD34+ DR- CD33- CD19- CD3- CD38-.

18. The method of claim 1 where in said target cells are selected from the group consisting of CD3+ cells, CD4+ cells, CD8+ cells, CD19+ cells, CD33+ cells, CD38+ cells and DR+ cells.

19. The method of claim 1 wherein said enriched fraction of target cells in said cultured second population comprises at least about 5% of said target cells.

20. The method of claim 1 wherein said enriched fraction of target cells in said cultured second population comprises at least about 20% of said target cells.

21. The method of claim 1 wherein said enriched fraction of target cells in said cultured second population comprises at least about 50% of said target cells.

22. The method of claim 1 wherein said enriched fraction of target cells in said cultured second population comprises at least about 80% of said target cells.

23. The method of claim 1 wherein said first cell population comprises whole peripheral blood.

24. The method of claim 1 wherein said first cell population comprises peripheral blood mononucleated cells.

25. The method of claim 24 wherein said peripheral blood mononucleated cells comprise a buffy-coat fraction.

26. The method of claim 24 wherein said peripheral blood mononucleated cells comprise a leukaphoresis fraction.

27. The method of claim 24 wherein said peripheral blood mononucleated cells comprise a FICOLL separation fraction.

28. The method of claim 24 wherein said peripheral blood mononucleated cells comprise immunoseparated fractions of desired leukocytes.

29. The method of claim 1 wherein said first cell population comprises peripheral blood mononucleated cells selected from the group consisting of freshly harvested cells and previously cryopreserved cells.

30. The method of claim 1 wherein said first and second cell populations are different cells.

31. The method of claim 1 wherein said first and second cell populations are the same cells.

32. The method of claim 1 wherein said first cell population comprises bone marrow cells.

33. The method of claim 1 wherein said first cell population comprises cord blood cells.

34. The method of claim 1 wherein said second cell population comprises whole peripheral blood.

35. The method of claim 1 wherein said second cell population comprises peripheral blood mononucleated cells.

36. The method of claim 35 wherein said peripheral blood mononucleated cells comprise a buffy coat fraction.

37. The method of claim 35 wherein said peripheral blood mononucleated cells comprise a leukaphoresis fraction.

38. The method of claim 35 wherein said peripheral blood mononucleated cells comprises a FICOLL separation fraction.

39. The method of claim 35 wherein said peripheral blood mononucleated cells comprise immunoseparated fractions of desired leukocytes.

40. The method of claim 1 wherein said second cell population comprises peripheral blood mononucleated cells selected from the group consisting of freshly harvested cells and previously cryopreserved cells.

41. The method of claim 1 wherein said second cell population comprises bone marrow cells.

42. The method of claim 1 wherein said second cell population comprises cord blood cells.

43. The method of claim 1 wherein said first and second cell populations are derived from the same donor.

44. The method of claim 43 wherein said first and second cell populations are derived from a single blood sample from said donor.

45. The method of claim 1 wherein said first and second cell populations are derived from different donors.

46. The method of claim 1 wherein the step of treating a first cell population comprises contacting said first cell population with an inducing agent to induce said first cell population to produce said BSFM.

47. The method of claim 46 wherein said inducing agent comprises a mitogen.

48. The method of claim 47 wherein said mitogen is a plant lectin.

49. The method of claim 48 wherein said lectin derived from a plant is selected from the group consisting of Phaseolus vulgaris, Dolichos biflorus, Solanum tuberosum, Sophora japonica, Maclura pomifera, Pisum sativum, Ulex europeus, Arachis hypogaea, Glycine max, Canavalia ensiformis, Triticum vulgaris, Lycopersicon esculentum, Phytolacca americana, and Listeria monocytogenes.

50. The method of claim 48 wherein said plant lectin is PHA or ConA.

51. The method of claim 47 wherein said mitogen is a T-cell mitogen.

52. The method of claim 51 wherein said T-cell mitogen is selected from the group consisting of Staphylococcal enterotoxin A, galactose oxidase, Streptolysin O protein A, and 12-O-tetradecanoylphorbol-13-acetate (TPA).

53. The method of claim 46 wherein said inducing agent is a monoclonal antibody to T cells.

54. The method of claim 53 wherein said monoclonal antibody is OKT3.

55. The method of claim 46 wherein said inducing agent is a diterpene ester.

56. The method of claim 55 wherein said diterpene ester is 12-O-tetradecanoylphorbol-13-acetate (TPA).

57. The method of claim 55 wherein said diterpene ester is selected from the group consisting of Phorbol (4-O-methyl) 12-myristate-13-acetate, Phorbol (20-oxo-20-deoxo) 12-myristate-13-acetate, Phorbol 12-monomyristate, Phorbol 12,13-didecanoate, Phorbol 12,13-dibutyrate, Phorbol 12,13-dibenzoate, and Phorbol 12,13-diacetate.

58. The method of claim 46 wherein said inducing agent is mezerein (MZN).

59. The method of claim 46 wherein said first population of cells is pretreated with an enhancing agent prior to contacting said first cell population with said inducing agent.

60. The method of claim 59 wherein said enhancing agent is selected from the group consisting of TPA, MZN, interferon-α (IFN-α) and interferon-β(IFN-β).

61. The method of claim 60 wherein said inducing agent is PHA and said enhancing agent is TPA.

62. The method of claim 1 wherein said target cell is CD34+ and said first cell population is treated by a pretreatment with an enhancing agent selected from the group consisting of TPA, MZN, IFN-α, IFN-β, and mixtures thereof and then contacted with an inducing agent selected from the group consisting of PHA, OKT3, Con A and mixtures thereof.

63. The method of claim 46 wherein said inducing agent and first cell population are combined in a growth medium and incubated for a period of from about 1 to 10 days.

64. The method of claim 63 wherein said inducing agent is added to the growth medium in an amount of from about 5 to 10 ug/ml of medium.

65. The method of claim 1 wherein said first cell population is treated in vitro to produce said BSFM.

66. The method of claim 1 wherein said first cell population is treated in vivo to produce said BSFM.

67. The method of claim 63 wherein said growth medium is a defined serum-free medium.

68. The method of claim 1 wherein said second cell population is cultured in a defined serum-free medium.

69. The method of claim 68 wherein said second cell population is cultured in the presence of from about 1% to about 10% of said BSFM by volume in said medium.

70. The method of claim 68 wherein at least one additional cytokine is added to said defined serum-free medium.

71. The method of claim 1 wherein said second cell population is cultured for about 5 to about 20 days and said target cells comprise CD34+ cells.

72. The method of claim 1 additionally comprising the step of maintaining the viability of the culture of said enriched second cell population.

73. The method of claim 1 additionally comprising the step of cryopreserving the enriched second cell population.

74. The method of claim 1 wherein said second cell population is cultured in vitro.

75. The method of claim 1 wherein said second cell population is cultured in vivo.

76. The method of claim 75 wherein said in vivo culture is effected by introducing said BSFM into a host.

77. The method of claim 75 wherein said in vivo culture is effected by introducing said cell population into a host after contacting said second cell population with said BSFM.

78. The method of claim 1 wherein said BSFM comprises a mixture of at least two factors selected from the group consisting of IFN-γ, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, CSF-G, CSF-GM, TNF-α, TNF-β, TGF-β, SCF, EPO, SCPF and BMPs.

79. A method for producing a cell population with an enriched fraction of at least one hematopoietic target cell which has a desired position in the hematopoietic chain comprising the steps of:

(a) treating a first cell population comprising hematopoietic cells to induce said first cell population to produce a balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from said first cell population and having a balance of stimulatory and inhibitory factors which preferentially favors the proliferation of pluripotent stem cells;

(b) modifying the composition of said BSFM to produce a modified balanced selective factor mixture having a new balance of stimulatory and inhibitory effects which preferentially favors the proliferation of said target cell; and

(c) culturing a second cell population comprising hematopoietic cells for a time sufficient and in a the presence of an amount of said modified BSFM sufficient to expand preferentially said target cells and enrich the fraction of said target cells in said second cell population.

80. A method for producing a cell population with an enriched fraction of at least one hematopoietic target cell which has a desired position in the hematopoietic chain comprising the steps of:

(a) treating a first cell population comprising hematopoietic cells to induce said first cell population to produce a balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from said first cell population and having a balance of stimulatory and inhibitory factors which preferentially favors the proliferation of at least one intermediate target cell selected from the group consisting of pluripotent stem cells, progenitor cells and mixtures thereof;

(b) culturing a second cell population comprising hematopoietic cells for a time sufficient and in a the presence of an amount of said BSFM sufficient to expand

preferentially said intermediate target cells and enrich the fraction of said intermediate target cells in said second cell population; and

(c) culturing said enriched cell population under conditions that favor the proliferation of said target cell to produce an expanded cell population with an enriched fraction of said target cell.

81. A method for producing an expanded hematopoietic cell population comprising at least about 20% of stem cells/progenitor cells expressing the CD 34 antigen from an original sample of donor peripheral blood comprising the steps of:

(a) treating a first cell population from said original sample with a T cell mitogen to induce said first cell population to produce a balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from said first cell population and having a balance of stimulatory and inhibitory effects which preferentially favors the proliferation of CD 34+ cells; and

(b) culturing a second cell population from said original sample for a time sufficient in the presence of an amount of said BSFM sufficient to expand preferentially the number of CD 34+ cells by a factor of at least 500-fold and provide an enriched cell population comprising at least about 20% CD 34+ cells.

82. A method for reconstituting the hematopoietic system in a host with a compromised hematopoietic system comprising transplanting into said host an expanded hematopoietic cell population enriched in selected target cells produced by the method of claim 2.

83. The method of claim 82 wherein the transplanted cell population is derived from said host prior to the event which compromised the hematopoietic system of said host.

84. The method of claim 82 wherein the transplanted cell population is derived from a donor having a matched HLA type.

85. The method of claim 82 wherein the transplanted cell population comprises pluripotent stem cells, progenitor cells or a mixture thereof.

86. The method of claim 1 wherein the transplanted cell population comprises CD34+ cells.

87. The method of claim 82 wherein said host is treated with an agent which increases the number of circulating peripheral blood mononucleated cells prior to the removal of hematopoietic cells from said host to process them for transplantation.

88. The enriched cell population produced by the process of claim 1.

89. The enriched cell population produced by the process of claim 79.

90. The enriched cell population produced by the process of claim 80.

91. The enriched cell population produced by the process of claim 81.

92. A balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from a hematopoietic cell population, said BSFM preferentially favoring the expansion and enrichment in culture of pluripotent stem cells.

93. A balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from a hematopoietic cell population, said BSFM preferentially favoring the expansion and enrichment in culture of cells selected from the group consisting of myeloid progenitors, lymphoid progenitors and mixture thereof.

94. A balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from a hematopoietic cell population, said BSFM preferentially favoring the expansion and enrichment in culture of cells selected from the group consisting of T cells, B cells, erythrocytes, monocytes, platelets, granulocytes, macrophages, basophils, eosinophils, the committed precursors of the foregoing and mixtures thereof.

95. The BSFM of claim 92 wherein said pluripotent stem cells are CD34+.

96. The BSFM of claim 94 wherein said T cells are selected from the group consisting of CD3+ cells, CD4+ cells and CD8+ cells.

97. The BSFM of claim 92 wherein said cell factors comprise at least two factors selected from the group consisting of IFN-γ8, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, CFS-G, CFS-GM, TNFα, TNF-β, TGFβ, SCF, EPO, SCPF and BMPs.

98. The method of claim 10 additionally comprising the step of culturing said erythrocytes and recovering the hemoglobin produced by said erythrocytes.

99. A method for producing a cell population with an enriched fraction of at least one hematopoietic target cell which has a desired position in the hematopoietic

claim comprising culturing a hematopoietic cell population for a time sufficient and in the presence of an amount of a balanced selective factor mixture (BSFM) having the active factors of the composition of the BSFM of claim 92, sufficient to expand preferentially said target cells and enrich the fraction of said target cells in said hematopoietic cell population.

100. A method for producing a cell population with an enriched fraction of at least one hematopoietic target cell which has a desired position in the hematopoietic claim comprising culturing a hematopoietic cell population for a time sufficient and in the presence of an amount of a balanced selective factor mixture (BSFM) sufficient to expand preferentially said target cells and enrich the fraction of said target cells in said cell population, said BSFM comprising a mixture of cell factors having a balance of stimulatory and inhibitory effects which preferentially favors the proliferation of said target cells and said BSFM having the active factors of a composition prepared by treating a first hematopoietic cell population with a mitogen to induce said first cell population to produce a mixture of cell factors which preferentially favors the proliferation of said target cells.

101. A method for selectively expanding a population of primary mammalian cells to provide an enriched fraction of target cells of a predetermined cell type comprising the steps of:

(a) treating a first primary cell population to induce said first cell population to produce a balanced selective factor mixture (BSFM) comprising a mixture of cell factors obtained from said first cell population and having a balance of stimulatory and inhibitory factors which preferentially favors the proliferation of said target cells; and

(b) culturing a second primary cell population for a time sufficient and in the presence of an amount of BSFM sufficient to expand preferentially said target cells and enrich the fraction of said target cells in said second primary cell population.

102. The method of claim 101 wherein said primary mammalian cell is selected from the group consisting of hematopoietic cells, liver cells, pancreas cells, skin cells, brain cells, nerve cells, lymph node cells, thymus cells, spleen cells, heart cells, bone

marrow cells, bone cells, cartilage cells, endothelial cells, kidney cells, muscle cells, and epithelial cells.

103. The method of claim 101 wherein the step of treating said first primary cell population comprises contacting said first cell population with a mitogen for said first cell population.

104. The process of claim 101 wherein said treating and culturing steps are conducted in vitro.

105. The method of claim 101 wherein said treating step is conducted in vivo.

106. The method of claim 101 wherein said culturing step is conducted in vivo.

107. The method of claim 106 wherein said culturing in vivo comprises tissue regeneration.

108. A method for creating a human stem cell bank comprising the steps of:

(a) removing a sample of hematopoietic cells from first donor of a known HLA type;

(b) preparing an expanded culture of said hematopoietic cells enriched in pluripotent stem cells according to the method of claim 2;

(c) cryopreserving said expanded culture; and

(d) repeating steps (a)-(c) for a separate donor of each known HLA type.

109. A method for performing an allogeneic stem cell transplant comprising introducing into a patient a stem cell population obtained by thawing a cryopreserved expanded culture produced by the method of claim 108 for the appropriate HLA type.

110. A method for ensuring the availability of sufficient quantities of healthy human stem cells for use by a patient who may need a stem cell transplant is the future comprising the steps of:

(a) preparing an expanded culture of hematopoietic cells enriched in pluripotent stem cells according to the method of claim 2 while said patient is healthy; and

(b) cryopreserving said enriched culture for future use by said patient.

111. The method of claim 82 wherein any abnormal or diseased hematopoietic cells are removed from said cell population prior to said transplanting.

112. A method for repairing damaged human tissue comprising the step of transplanting into a patient an expanded primary cell population of the type of said damaged tissue, said expanded primary cell population being prepared by the method of claim 101.

113. A method for regenerating a human organ comprising introducing one or more expanded primary cell populations prepared according the method of claim 101 into a suitable structural matrix.

114. A method for the noninvasive determination of fetal genetic disorders comprising the steps of:

(a) removing a sample of peripheral blood from a pregnant woman comprising a hematopoietic cell population containing circulating fetal cells;

(b) expanding said hematopoietic cell population and enriching the fraction of both fetal and maternal cells by the method of claim 1;

(c) separating said fetal cells from said maternal cells; and

(d) performing genetic analysis on said fetal cells.

115. A method of providing a prophylactic treatment of hematopoietic cells to a patient comprising the steps of:

(a) preparing an expanded hematopoietic cell population enriched in target cells of prophylactic value by the method of claim 1; and

(b) introducing a prophylactically effective amount of said cell population into said patient.

116. The method of claim 115 wherein said target cells of prophylactic value comprise T cells.

117. The method of claim 116 wherein said T cells are selected from the group consisting of CD4+ cells and CD8+ cells.

118. A method for treating a patient, comprising the steps of:

(a) providing an original cell population comprising hematopoietic cells;

(b) culturing a least a portion of said original cell population for a time sufficient and in the presence of a balanced selective factor mixture (BSFM) comprising a mixture of cell factors prepared by treating a second cell population comprising hematopoietic cells to induce said second cell population to produce said BSFM having a balance of stimulatory and inhibitory factors which preferentially favors the proliferation of at least one target cell of a desired position in the hematopoietic chain, said BSFM being present in an amount sufficient to expand preferentially said target cells and enrich the fraction of said target cells in said original cell population; and

(c) introducing at least a portion of said enriched cell population into said patient.

119. The method of claim 118 wherein said original cell population and said second cell population are from the same patient.

120. The method of claim 119 wherein said second cell population is a portion of said original cell population are from said patient.

121. The method of claim 118 wherein said second cell population is from a different individual.

122. The method of claim 121 wherein said different individual is an identical twin to said first individual.

123. The method of claim 121 wherein said different individual has matched HLA type.

124. The method of claim 118 wherein said patient has an immune system disorder.

125. The method of claim 118 wherein said patient is immunodeficient.

126. The method of claim 125 wherein said immunodeficiency is due to a genetic defect.

127. The method of claim 126 wherein said genetic defect causes SCID.

128. The method of claim 125 wherein said immunodeficiency is due to a pathogenic infection.

129. The method of claim 128 wherein said patient is infected with HIV.

130. The method of claim 125 wherein said immunodeficiency is due to treatment of the patient with ablative chemotherapy or radiation therapy.

131. The method of claim 124 wherein said patient has an autoimmune disorder.

132. The method of claim 118 wherein said patient has a hemolytic disorder.

133. The method of claim 118 wherein said patient has a congenital hematopoietic disorder.

134. The method of claim 133 wherein said patient has a genetic defect.

135. The method of claim 133 wherein said patient has a disorder selected from the group consisting of Fanconi's anemia, Thalassemia major, and Lysomal storage disease.

136. The method of claim 132 wherein said patient has aplastic anemia.

137. The method of claim 118 wherein said patient has a malignancy disorder.

138. The method of claim 137 wherein said patient has a hematopoietic malignancy.

139. The method of claim 138 wherein said patient has a disorder selected from the group consisting of leukemias, lymphomas and myelomas.

140. The method of claim 137 wherein said patient has a solid tumor.

141. The method of claim 140 wherein said patient has a tumor selected from the group consisting of breast, neural, ovarian and testicular carcinomas.

142. The method of claim 118 wherein said treatment additionally comprises the step of stably incorporating into the genome of at least or part of said introduced cell population a gene sequence that encodes for the expression of therapeutic gene products.

143. The method of claim 142 wherein said therapeutic gene product supplies deficiencies of a defective gene.

144. The method of claim 142 wherein said therapeutic gene products are toxic to infection-causing pathogens.

145. The method of claim 142 wherein said gene sequence is antisense to and prevents the expression of undesirable gene products.

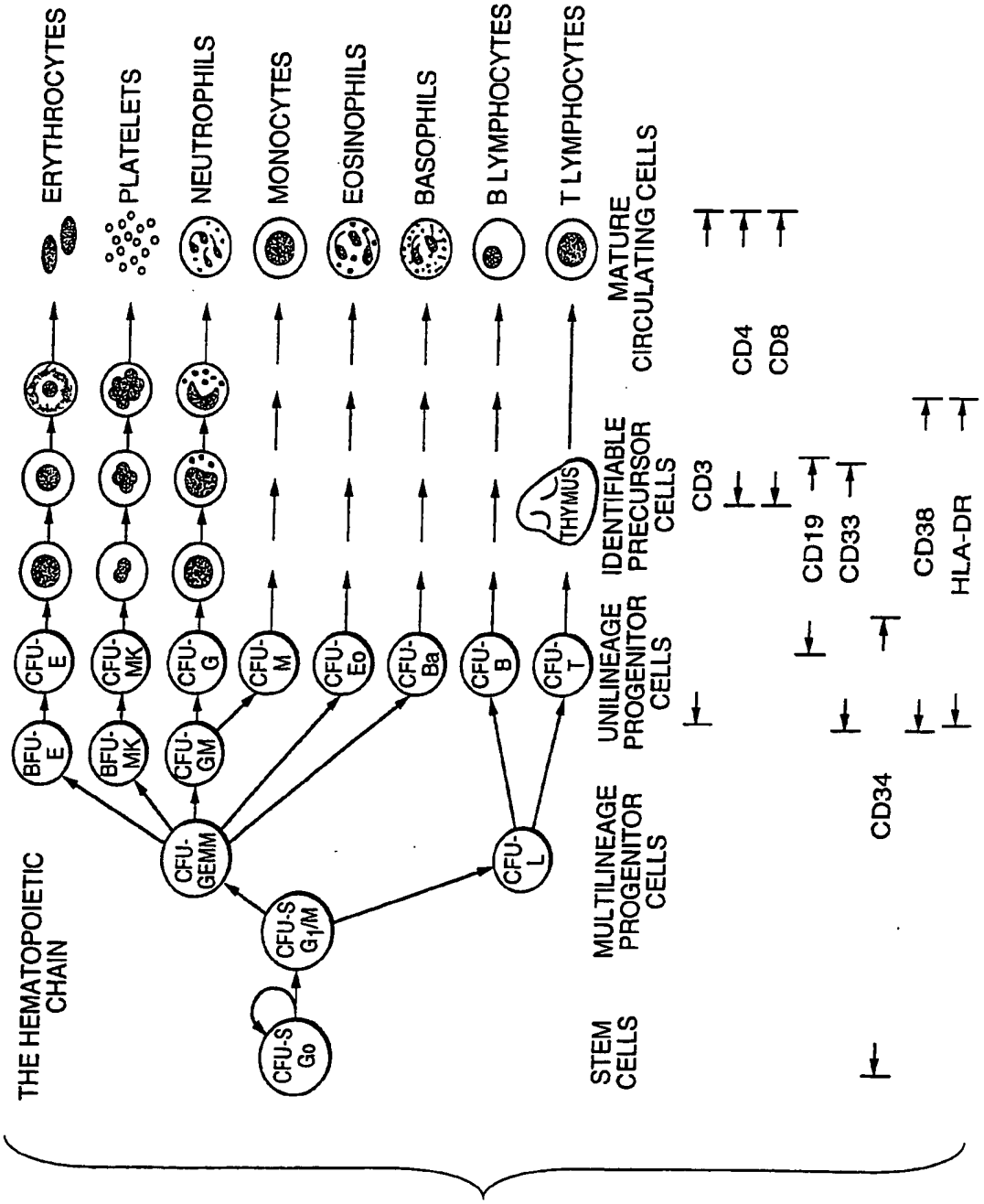
146. The method of claim 118 wherein said original cell population is selected from the group consisting of peripheral blood mononucleated cells, bone marrow cells and cord blood mononucleated cells.

147. The method of claim 118 wherein said target cell is selected from the group consisting of pluripotent stem cells, progenitor cells, precursor cells, B cells, T cells, monocytes, granulocytes, erythrocytes, platelets, macrophages, eosinophils, basophils and mixtures thereof.

148. The method of claim 118 wherein said culturing step is performed in vitro.

149. The method of claim 118 wherein said original cell population is provided by explanting the cells from said patient.

150. The method of claim 118 additionally comprising the steps of cryopreserving at least a portion of said original cell population or said enriched cell population and thawing out said population prior to its use.


THE HEMATOPOIETIC CHAIN
STEM CELLS
CFU-S Go
CFU-S G1/M
MULTILINEAGE PROGENITOR CELLS
CFU-GEMM
CFU-L
UNILINEAGE PROGENITOR CELLS
BFU-E
CFU-E
BFU-MK
CFU-MK
CFU-GM
CFU-G
CFU-M
CFU-Eo
CFU-Ba
CFU-B
CFU-T
IDENTIFIABLE PRECURSOR CELLS
THYMUS
MATURE CIRCULATING CELLS
ERYTHROCYTES
PLATELETS
NEUTROPHILS
MONOCYTES
EOSINOPHILS
BASOPHILS
B LYMPHOCYTES
T LYMPHOCYTES
CD3
CD4
CD8
CD19
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CD34
CD38
HLA-DR

FIG. 1

FIG. 2

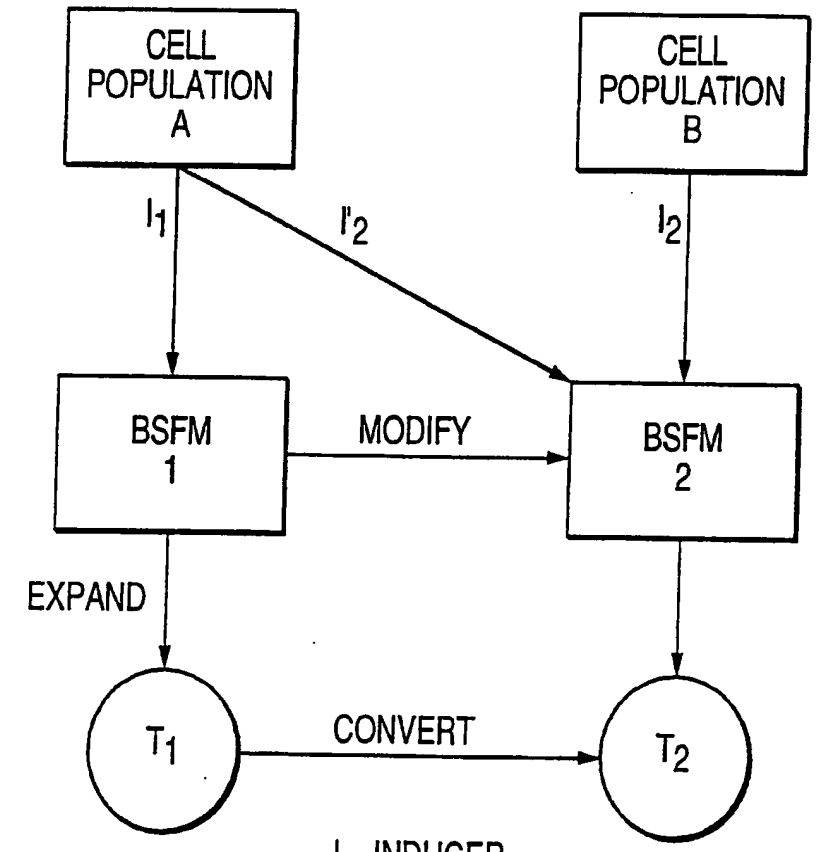

CELL POPULATION A
CELL POPULATION B
I_1
I'_2
I_2
BSFM 1
MODIFY
BSFM 2
EXPAND
T_1
CONVERT
T_2
I = INDUCER
BSFM = BALANCED STEM CELL PROLIFERATION MIXTURE

FIG. 3

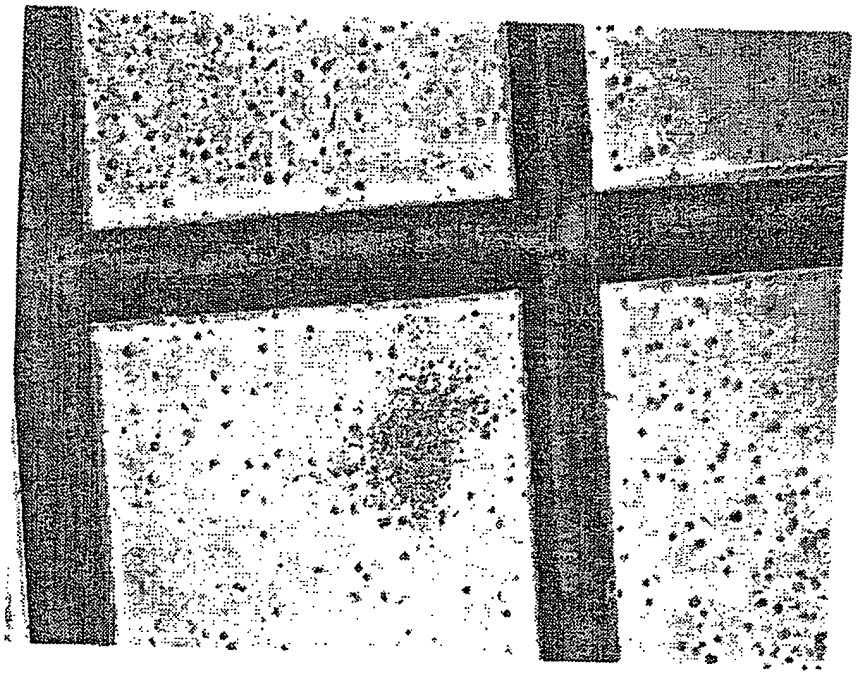


FIG. 4

FIG. 5

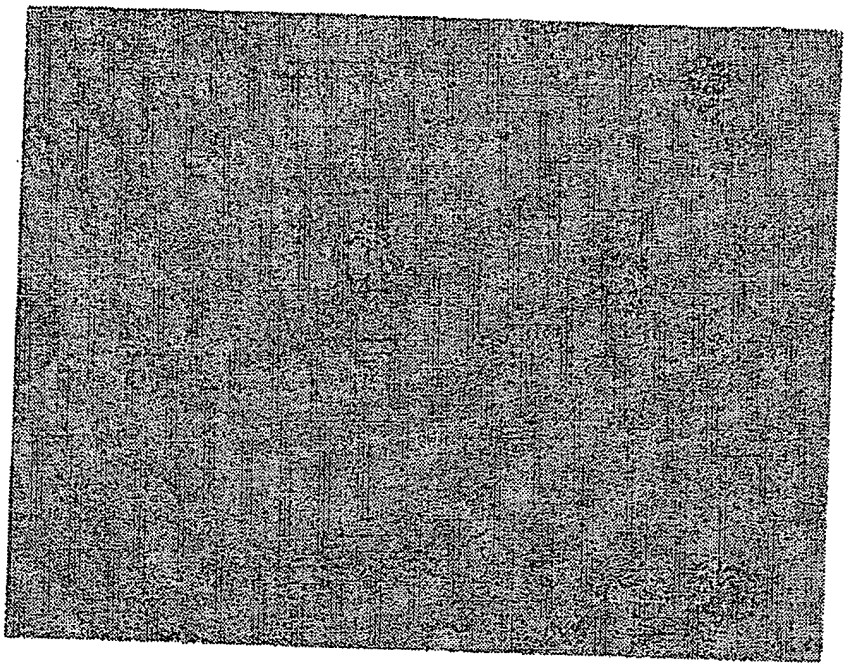


FIG. 6

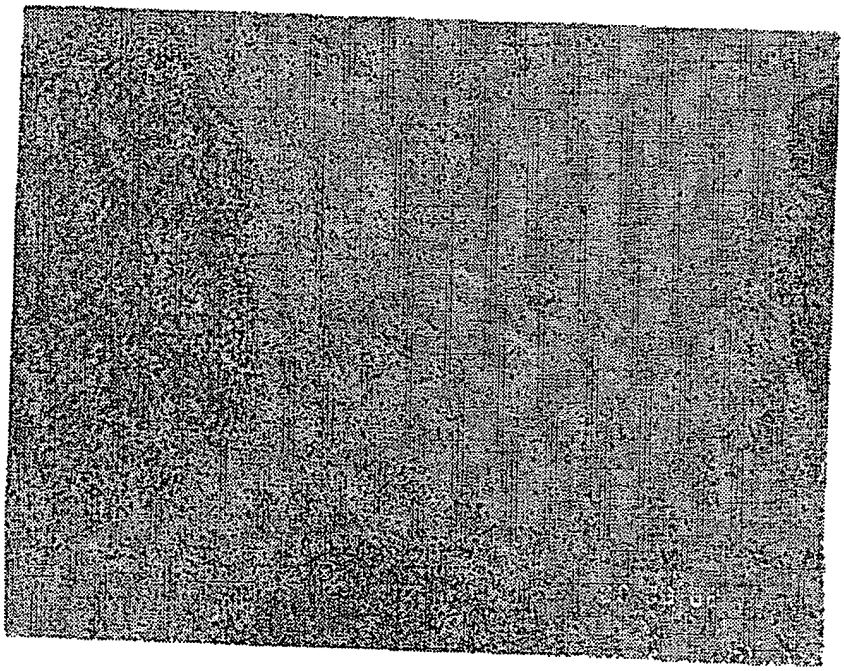


FIG. 7

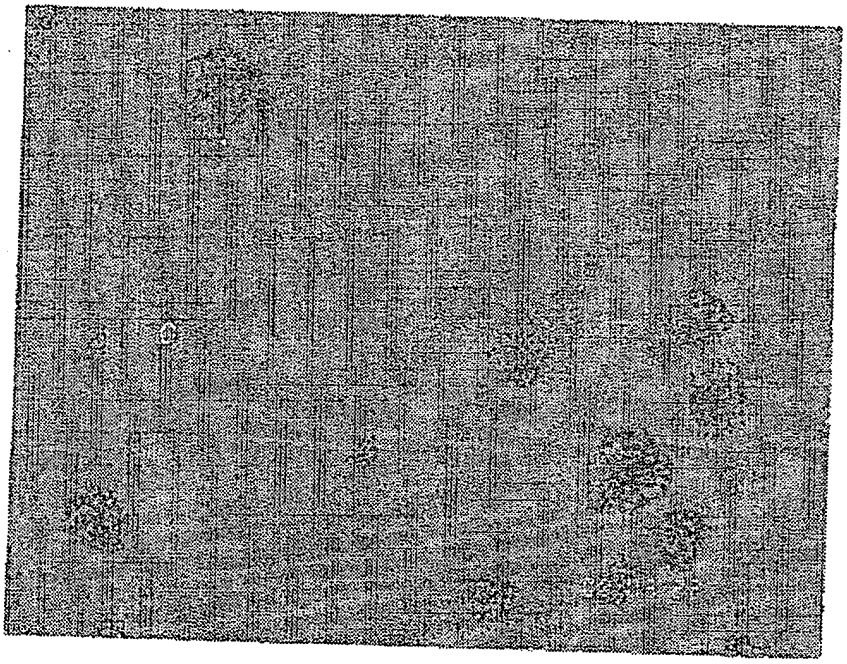
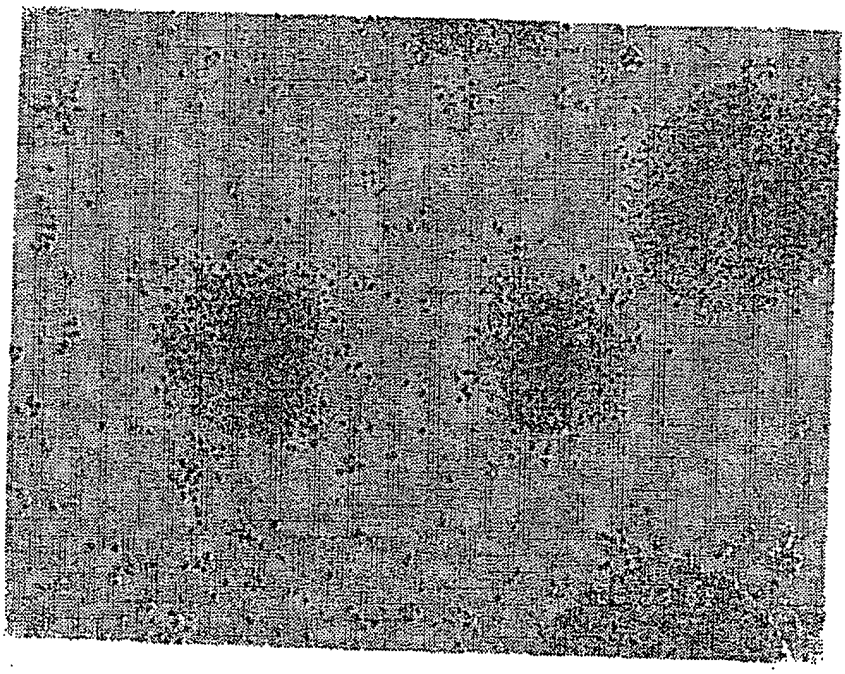
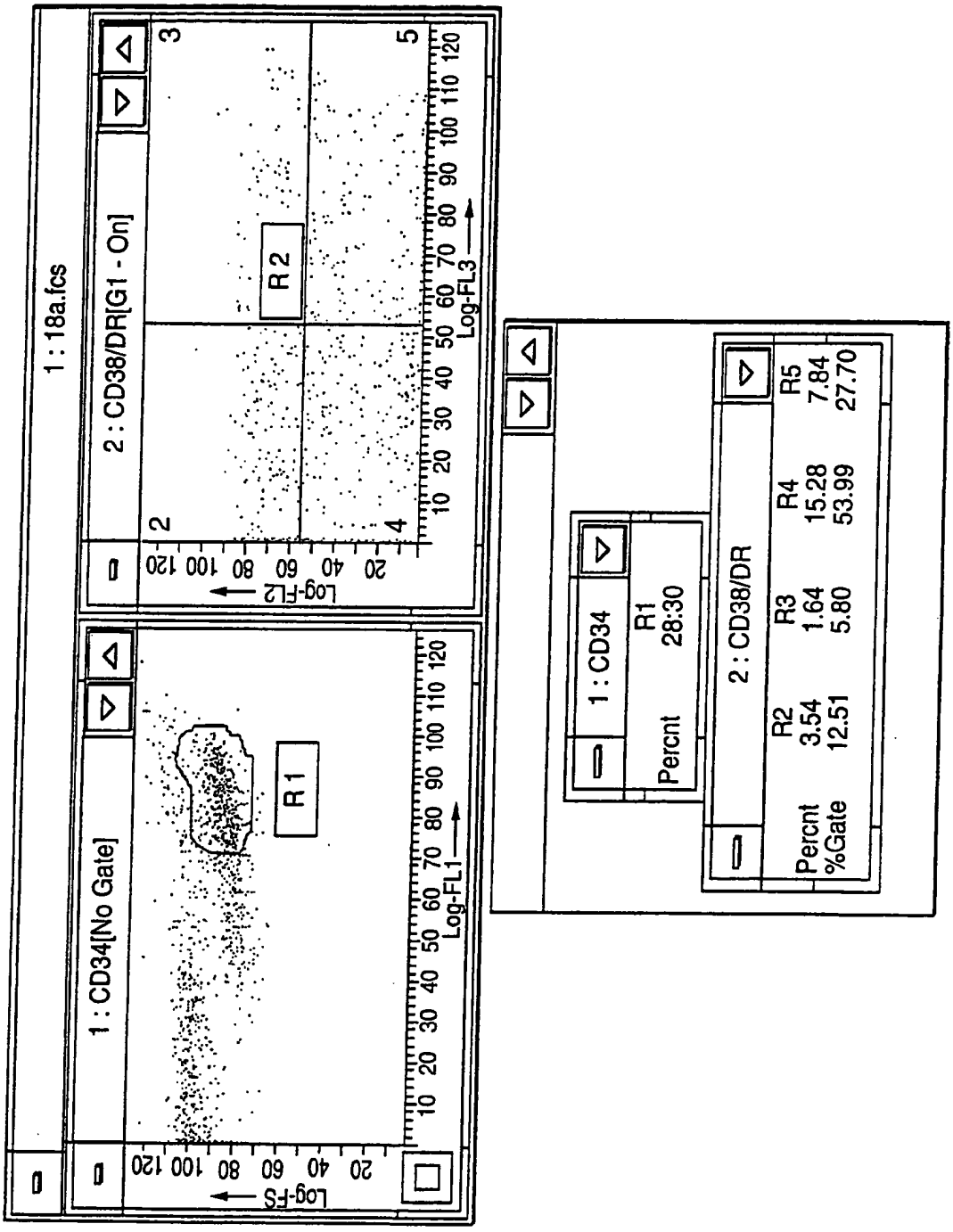


FIG. 8

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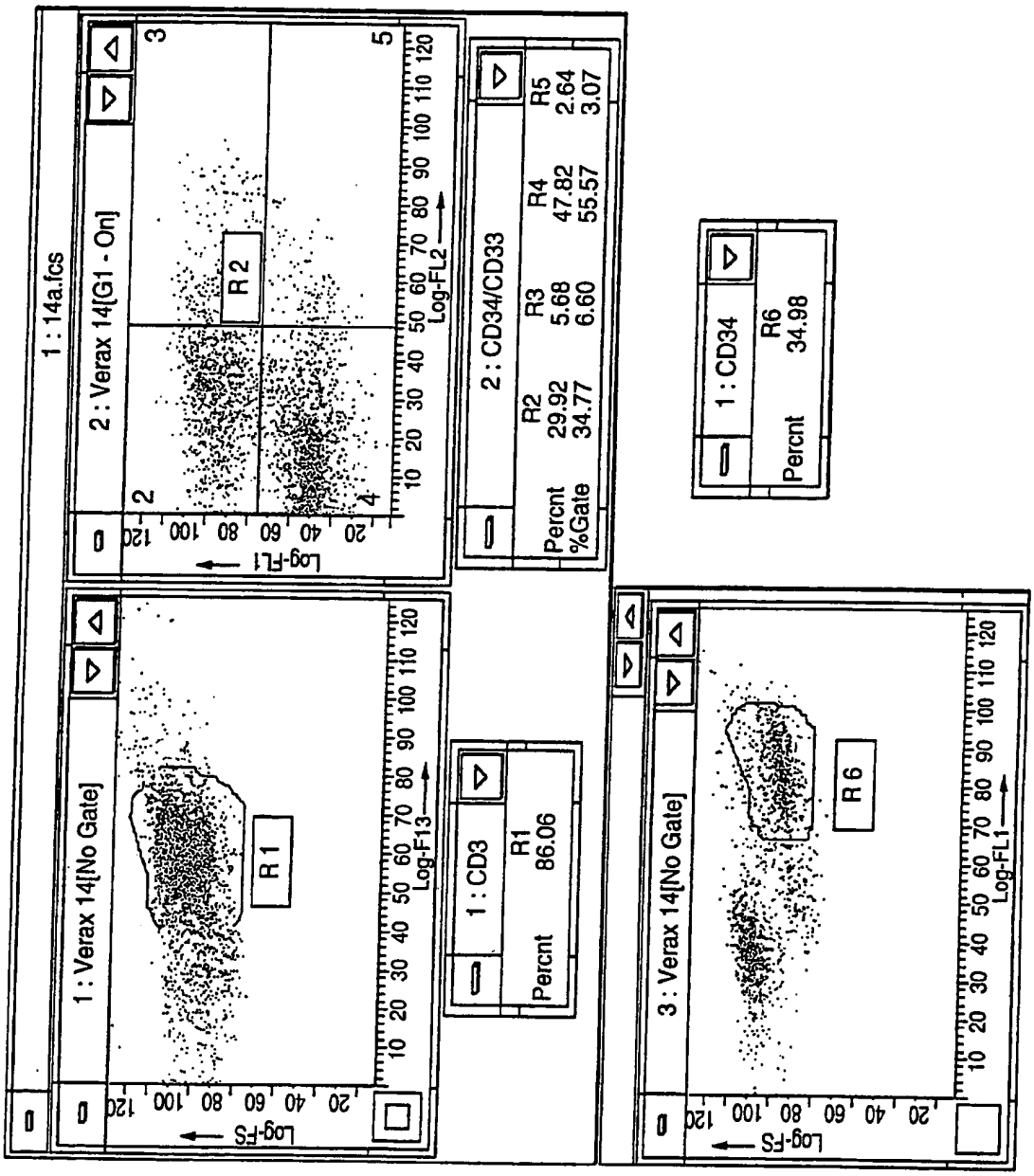
2 : CD38/DR[G1 - On]
R2
Log-FL2
Log-FL3
1 : CD34[No Gate]
R1
Log-FS
Log-FL1
1 : CD34
R1
28:30
Percnt
2 : CD38/DR
R2 R3 R4 R5
Percnt 3.54 1.64 15.28 7.84
%Gate 12.51 5.80 53.99 27.70

FIG. 9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R 2
Log-FL1
Log-FL2
2 : CD34/CD33
R2 R3 R4 R5
Percnt 29.92 5.68 47.82 2.64
%Gate 34.77 6.60 55.57 3.07
1 : CD34
R6
Percnt 34.98
1 : Verax 14[No Gate]
R 1
Log-FS
Log-F13
1 : CD3
R1
Percnt 86.06
3 : Verax 14[No Gate]
R 6
Log-FS
Log-FL1

FIG. 10

FIG. 11

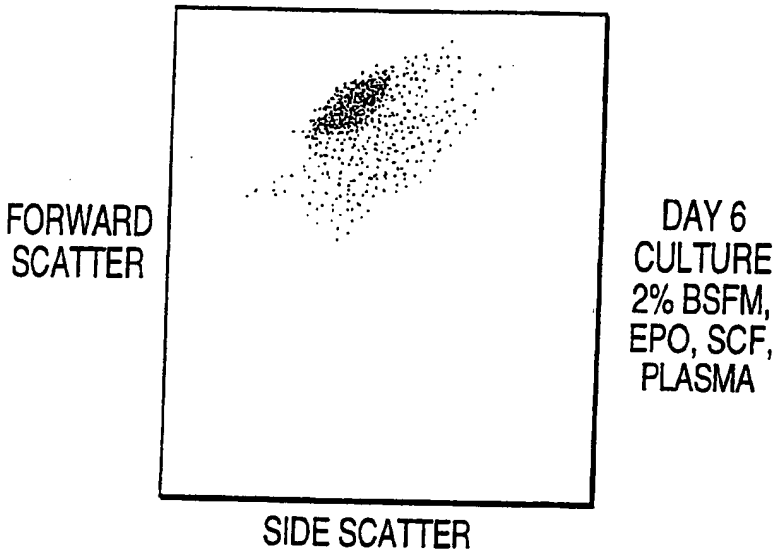
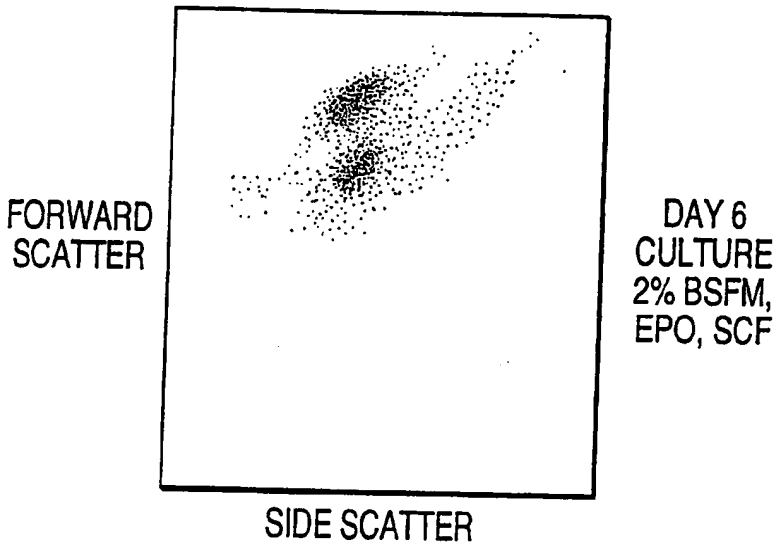

FORWARD SCATTER
DAY 6 CULTURE 2% BSFM, EPO, SCF, PLASMA
SIDE SCATTER

FIG. 12


FORWARD SCATTER
DAY 6 CULTURE 2% BSFM, EPO, SCF
SIDE SCATTER

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01033

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93; 435/2, 240.1, 240.2, 240.21, 240.25, 240.3, 240.31; 514/44, 2; 530/300, 350, 829

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Annual Review of Immunology, Volume 1, issued 1983, C. G. Fathman et al., "T-lymphocyte clones", pages 633-655, see entire document.	1, 30, 35, 46-48, 50, 51, 65, 74, 78, 99, 100
Y	D.W. Golde, "Hematopoiesis" published 1984 by Churchill Livingstone (N.Y.), pages 73-179, see entire document.	1-150
Y	Blood Cells, Volume 17, issued 1991, E.F. Srour et al., "Human CD34+ HLA-DR- bone marrow cells contain progenitor cells capable of self-renewal, multilineage differentiaition, and long-term in vitro hematopoiesis", pages 287-295, see entire document.	1, 2, 13, 14-74, 78, 79, 81, 94, 100

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
28 APRIL 1994	09 MAY 1994

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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Clinical Investigation, Volume 82, issued September 1988, J. Brandt et al., "Characterization of a human hematopoietic progenitor cell capable of forming blast cell containing colonies in vitro", pages 1017-1027, see entire document.	1, 2, 13, 14-74, 78, 79, 81, 99, 100
Y	Blood, Volume 74, Number 5, issued October 1989, H.J. Sutherland et al., "Characterization and partial purification of human marrow cells capable of initiating long term hematopoiesis in vitro", pages 1563-1570, see entire document.	1, 2, 13, 14-78, 79, 81, 99, 100

INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US94/01033

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 35/12, 37/00, 48/00; A01N 1/02; C12N 5/00, 5/02, 5/06, 5/08, 5/10; C07K 7/00, 13/00, 15/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/93; 435/2, 240.1, 240.2, 240.21, 240.25, 240.3, 240.31; 514/44, 2; 530/300, 350, 829

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, BIOSIS, CHEM AB, DERWENT WPI, EMBASE, PASCAL, LSC, search terms: author names, cells, bsfm, conditioned media, cas, conA, pha, scf, stem cell factor, c kit, steel factor, mgf, cona supernatants, T cell, B cell, macrophage, leukocytes, lectin, mitogen, tpa, lymphokine, cytokine, lymphocytes